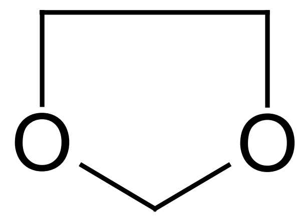

1,3-DIOXOLANE

CAS Number 646-06-0

USEPA HPV Challenge Program Submission

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Executive Overview

1,3-Dioxolane (Dioxolane) is a stable reaction product of ethylene glycol and formaldehyde used primarily as a monomer for production of high-molecular weight polyacetals. Other uses are as a chemical intermediate, process solvent and stabilizer for halogenated solvents. It is a volatile liquid that is miscible with water in all proportions. Dioxolane has a production volume exceeding one million pounds per annum; hence, it was listed by the Environmental Protection Agency on its list of chemicals in the High Production Volume Chemical Challenge Program. This document is Industry's response to the EPA challenge to define the toxicologically relevant data set within the HPV guidelines and fill gaps in our knowledge.

The physicochemical properties of Dioxolane are well defined. High water solubility and moderate volatility are defining characteristics. Environmental fate data are available from a combination of experimental and modeling data. Dioxolane is not readily biodegradable, is stable in water for over at year in the pH 4 to 9 range and has an estimated photodegradation half-life in air in the range of 10 to 30 hours. Predicted values for fugacity have been calculated with the MacKay model, which indicate an initial primary distribution in water; however, experience shows that in open systems it rapidly volatilizes to air where it will be destroyed by photooxidation. Fish, daphnia and green algae are only acutely affected by Dioxolane at concentration levels greater than several hundred ppm. Dioxolane demonstrates a low order of acute toxicity to mammals by the oral, inhalation and dermal routes. Genotoxicity has been evaluated using multiple *in vitro* and *in vivo* experimental procedures covering both mutation and chromosome aberration. The weight of evidence indicates lack of significant genotoxic properties. Reliable repeat-dose studies have been conducted by the oral and inhalation routes and a high-reliability 13-week inhalation study is available. The blood forming system was found to be the most sensitive target organ with a clear 13-week NOAEL of 300 ppm by inhalation. Reproductive toxicity has been evaluated in one-generation drinking water and inhalation studies. Adverse reproductive effects are absent at dosage levels below maternally toxic doses. Developmental toxicity results are available indicating that Dioxolane is not a specific developmental toxin.

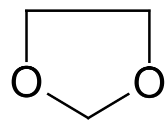
No additional testing is required under the EPA HPV Challenge Program as all relevant parameters are sufficiently characterized by the existing data and acceptable modeling. The aquatic stability and poor biodegradation in an OECD 301 ready biodegradation test indicate exposure of humans and aquatic organisms by way of the aqueous compartment would be the only area of potential concern. Data from the manufacturers, however, indicate that the level of Dioxolane being released from waster treatment plants is below the limit of detection or very low relative to its effect level. This suggests degradation in waste treatment plants and, coupled with the low toxicity, indicate low potential for harm by this route. Industrial exposures are two orders of magnitude below the subchronic NOEL and consumer exposures are negligible. There is a low priority for additional work on Dioxolane.

Test Plan in Table Format

CAS Number 646-06-0 1,3-Dioxolane	Information	OECD Study	GLP Study	Other Study	Estimation	Acceptable	Testing Required
HPV Endpoint							
Physical Chemical							
Melting Point	Y	N	N	N	N	Y	N
Boiling Point	Y	N	N	N	N	Y	N
Vapor Pressure	Y	N	N	Y	N	Y	N
Water Solubility	Y	N	N	N	N	Y	N
Partition Coefficient	Y	N	N	N	N	Y	N
Environmental & Fate							
Photo-Degradation	Y	N	N	N	Y	Y	N
Water Stability	Y	Y	Y	N	N	Y	N
Transport	Y	N	N	N	Y	Y	N
Biodegradation	Y	Y	Y	N	N	Y	N
Ecotoxicity							
96-Hour Fish	Y	Y	Y	Y	N	Y	N
48-Hour Invertebrate	Y	Y	Y	Y	N	Y	N
72-96-Hour Algae	Y	Y	Y	Y	N	Y	N
Toxicity							
Acute Oral	Y	N	N	Y	N	Y	N
Acute Inhal	Y	N	N	Y	N	Y	N
Acute Dermal	Y	N	N	Y	N	Y	N
Repeated Dose	Y	Y	Y	Y	N	Y	N
Reproductive	Y	N	N	Y	N	Y	N
Developmental	Y	Y	Y	Y	N	Y	N
Genetic Toxicology *in vitro*	Y	N	N	Y	N	Y	N
Genetic Toxicology *in vivo*	Y	N	Y	Y	N	Y	N

Introduction

1,3-Dioxolane, CAS Number 646-06-0, (Dioxolane) is a cyclic reaction product of ethylene glycol and formaldehyde. It is a volatile liquid miscible with water in all proportions. Its structure is:



Use Pattern

The primary uses for Dioxolane are:

- Co-monomer for manufacture of polyacetals and other polymers.
- Solvent for chemical reactions (including inorganic salts).
- Stabilizer for halogenated organic solvents
- As a starting material or reagent for organic synthesis

Among these potential uses 95% or more of the current production is consumed in either the production of polyacetals or as a starting material for the production of a drug substance. Almost all of the remainder is used as a process solvent. There are currently no consumer applications for Dioxolane. Thus, all exposure occurs in industrial settings where the material is a desired reaction product or used as a reactant or solvent. In these circumstances, exposures are well controlled by standard industrial hygiene practices. The majority end product of Dioxolane, Polyacetals, are very high molecular weight compounds of little toxicological concern. This plastic is strong and ridged and replaces metals in many engineering applications, especially where low-friction properties are important. The polymer contains a very low level of free unpolymerized Dioxolane and consumer exposure represents a negligible risk. FDA has approved polyacetal for food contact use and NSF has approved it for potable water.

Exposure Information

The limited use pattern for Dioxolane reduces the potential for exposure of humans or environmental species. Human exposure is thought to be limited to production workers involved in the manufacture of Dioxolane, the production of polyacetals, or the use of Dioxolane as a chemical intermediate. Exposure data from industrial hygiene monitoring of production and polyacetal manufacturing areas from a major producer indicates that worker exposure levels are low. In a period of several years of monitoring, data from 91 separate measurements of air concentration (time-weighted averages of 6 to 12 hour monitoring) showed an average level of 0.29 ± 0.39 ppm. The range of values was from 0 to 1.6 ppm. Another producer conducts annual monitoring and the value has been less than 1 ppm (the level of sensitivity) over the lifetime of the monitoring program.

Environmental releases of Dioxolane could occur from released vapors or from wastewater effluent. Measurements of released vapor concentration are not available, but vapor release is considered minimal based on levels measured in the workplace. Wastewater has been monitored for releases and Dioxolane concentration has been found to be below the limit of detection (0.1 ppm) in one plant. In another plant, it was found to average about 4 ppm with a higher release just after startup of production and Dioxolane release falling to below the limit of detection (1 ppm) after the start-up period. All known users of commercial Dioxolane have an approved wastewater treatment facility on site. Thus, aquatic releases are considered minimal.

Synonyms

- CASRN: 646-06-0
- 1,3-Dioxacyclopentane
- 1,3-Dioxolan
- Dioxolane
- 1,3-Dioxole, dihydro-
- Ethylene glycol formal
- Formal glycol
- Glycolformal
- Glycol methylene ether

Physical-chemical Data (HPV)

Physical-chemical data for Dioxolane are available from the literature and company sources.

Melting Point	-95 deg C [1]
Boiling Point	78 C @ 765 mm Hg[1]
Vapor Pressure	70 mm at 20 deg C.[2] 79 mm at 20 deg C.[3]
Partition Coefficient	log $K_{o/w}$ = - 0.37 [4]
Water Solubility	Soluble in all proportions[1]

Summary: Dioxolane is a volatile water-soluble liquid that distributes preferentially into water over n-octanol.

Recommendation: Dioxolane is sufficiently well characterized regarding these parameters for the purposes of hazard and risk assessment. No additional work is recommended.

Miscellaneous Properties (non-HPV)

• Empirical formula	$C_3H_6O_2$
• Molecular Weight	74.09
• Flash Point:	35 deg F (1.67 deg C) (Open Cup)[2]
• Density/Specific Gravity:	1.0600 @ 20 deg C
• Bulk Density:	8.2 lb/gal @ 20 deg C.[2]
• Solubilities:	Soluble in water, alcohol, ether, acetone

Environmental Fate and Pathways

Photodegradation

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals.[5] The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. In the case of Dioxolane, the APOWIN estimate for the reaction rate is based on simple hydrogen abstraction. Compounds similar to Dioxolane give a good correlation between estimates and measured values for this rate constant. Thus, this method is expected to provide an accurate estimate of the reaction rate constant with hydroxyl radical. Using this estimated value and the default in APOWIN for atmospheric hydroxyl radical concentration, the estimated half-life is approximately 11.5 hours.

Another estimate for atmospheric fate is reported in the National Library of Medicine's Hazardous Substance Data Base file (HSDB) as follows. "Based on the vapor pressure of 79 mm Hg at 20 deg C, 1,3-dioxolane is expected to exist almost entirely in the vapor phase in the ambient atmosphere. Vapor phase 1,3-dioxolane is degraded in the ambient atmosphere by reaction with photochemically formed hydroxyl radicals; the half-life for this reaction in air can be estimated to be about 1.1 days.[6]" The reference used in this HSDB entry is to the Atkinson SAR method for estimation of the rate constant for the reaction of various organic chemicals with hydroxyl radicals in the gas phase. This estimate varies only slightly from that obtained with the current APOWIN program in EPIWIN[5].

Water Stability

Water stability has been determined for Dioxolane using an OECD 111 compliant protocol. In this study, Dioxolane was incubated for four days at pH 4, 7 and 9 at 51˚ C. Hydrolysis was below five percent at all pH values. Accordingly, Dioxolane is estimated to be stable in the aquatic environment under typical environmental conditions for over one year.[7] It should be recognized that volatilization would occur and result in loss of Dioxolane if the aquatic system is exposed to air.

Theoretical Distribution

Theoretical Distribution (Fugacity) was estimated using the MacKay (Level III) model with standard defaults contained in EPIWIN v 3.05. The results for distribution using a model calculated $K_{o/c}$ of 0.175 are:

- Air 4.1 %
- Water 54 %
- Soil 42 %
- Sediment 0.1 %

Biodegradation

A recent Closed–Bottle Test (OECD 301D) indicates that Dioxolane is not considered readily biodegradable, consuming only 3.7% of the theoretical oxygen demand after 35 days of incubation. Positive and negative controls in this study were in the normal range.[8]

A screening level BOD_{15} study sponsored by Celanese provides support for the closed bottle test. In this study using municipal secondary sludge, the BOD_{15} was 0.063 mg oxygen taken up per mg compound. This indicates a 12% biodegradation in 15 days for dioxolane. The BOD_{10} was reported to be 0.035 mg suggesting that moderate to slow degradation was occurring under these conditions.[9]

Volatilization from surface waters is expected to be a significant means of loss from the aquatic compartment. This estimate is based on the experimentally derived Henry's Law Constant of 2.4×10^{-5} atm-cu m/mole at 25˚C.[10] The experimental Henry's Law Constant is also given in the EPIWIN program as 2.45×10^{-5} atm-cu m/mole and referenced as Cabani et al.[11] Both of these experimental values are in accord with the estimate of 2.23×10^{-5} atm-cu m/mole calculated by the HENRYWIN v3.1 program found in EPIWIN.[5] The extent of biotic and abiotic degradation has been established to be low since Dioxolane is stable in water and does not readily biodegrade.

The log $K_{o/w}$ of –0.37 suggests low bioaccumulation potential.

Summary: Dioxolane is chemically stable in the aquatic environment. It does not readily biodegrade and the primary fate for material entering the environment is photodegradation by atmospheric hydroxyl radical. The high volatility and magnitude of the Henry's Law constant suggest that Dioxolane will efficiently volatilize from open systems and undergo atmospheric photo-oxidation. The Octanol-water partition coefficient indicates that it has low potential for bioaccumulation.

Recommendation: Physical constants necessary for estimation of distribution of Dioxolane in the environment are well known and no additional work is recommended for physical constants. Regarding fate in the environment, no new testing is required under HPV. Although Dioxolane is hydrolytically stable and not readily biodegradable, the manufacturers find that the Dioxolane is either undetectable (limit of detection 0.1 ppm) in waste-treatment plants or reduced by about 85% by treatment[12 13]. These data suggest that Dioxolane is to a great extent removed by an acclimated wastewater treatment facility and, since it has demonstrated low toxicity to aquatic organisms, no additional testing is recommended.

Ecotoxicity

Fish

Dioxolane was found to have a low order of toxicity to typical aquatic environmental species. The LC_{50} (96 -hour) for freshwater fish was found to be greater than 95.4 mg/l in a recent OECD 203 guideline compliant study.[14] The LC_0 and the NOEC were found to be 95.4 mg/l. The study was conducted as a static-renewal study with daily renewal of test solution to prevent loss due to volatilization. No mortality was observed in this limit test.

A screening level study in saltwater fish, sponsored by Celanese, was conducted in which Sheepshead minnows (*Cyprinodon variegates, five* per group) were exposed to Dioxolane at concentrations of 7500, 11000, 13000, 15000 and 25000 mg/l. In this study, the 48=hourLC_{50} was reported to be 12000 mg/l and the 96-hour LC_0 was 7500 mg/l, the 96-hour LC_{50} was reported to be 10000 mg/l. A clear dose-response was established with a 24-hour mortality of 5/5 at 25000 mg/l.[9] Based on the volatility of Dioxolane, the actual value for the EC_{50} in this study is likely lower than reported since the actual average concentration was probably lower than the nominal concentration. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward bluegill sunfish in the OECD 203 guideline study. Other support for a low-level of toxicity comes from modeling. Using the EPA ECOSAR model found in EPIWIN the 96-hour estimated LC_{50} for fish is 8150 mg/l, a value close to that reported in the screening-level study.[5]

Aquatic Plants

Adverse effects on aquatic plants were examined in two studies. The first is a recent OECD 201 compliant study in which the E_bC_{50} and E_rC_{50} (0-72 hours) were >877 mg /l (based on measured concentrations). The 72-hour no-observable-effect concentration (NOEC) was 877 mg/l.[15] The 877 mg/l concentration was the highest level tested. The second study is an older screening level study in which Dioxolane was tested for growth inhibition of *Selenastrum capricornutum*. In this study, algae growth was measured out to 14 days past initial exposure at levels of 1000, 5000 or 10000 mg/l with counts recorded on day 3 and later. Significant inhibition was seen only at 5000 mg/l and above. In this study, 1000 mg/l was determined to be the NOEC.[9] The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour EC_{50} for green algae to be 4075 mg/l.[5]

Aquatic Invertebrates

Daphnia toxicity was evaluated in a definitive OECD guideline study and in a screening-level study. In the first, which followed the OECD 202 guideline, the EC_{50} (48 hour) was >772 mg/l under static renewal conditions to ensure exposure to representative concentrations of Dioxolane.[16]

The second study is a screening level study, in which Dioxolane was tested at 1000, 5000, 6500, 8000, 9000, 10000 or 12500 mg/l. In this study, the 24-hour EC_{50} was reported to be 7650 mg/l and the 48-hour EC_{50} was reported to be 6950 mg/l. Twenty daphnids were exposed and a clear dose-response relationship was obtained.[9] Based on the volatility of Dioxolane, the true value for the EC_{50} in this study may be lower than reported. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward daphnids. Using the EPA ECOSAR model, found in EPIWIN, the 48-hour estimated LC_{50} for daphnia is 7,400 mg/l.[5]

Summary: The ecotoxicity results clearly indicate that Dioxolane is of low concern to aquatic environmental species. In addition, the volatility of Dioxolane would limit the level of material found in surface water.

Recommendation: The toxicity of Dioxolane to aquatic organisms is sufficiently well established. The high volatility limits the extent to which terrestrial organisms will be exposed to Dioxolane. No additional testing is required.

Health Elements

Acute Toxicity

Oral Route

The oral LD_{50} in the rat was determined to be 5,200 mg/kg in a 1980 study sponsored by Celanese Corporation.[17] This was a five-dose study and the clinical signs associated with gavage exposure are well documented. In another study found in the literature, Czajkowska, et al.[18] reported the oral LD_{50} of Dioxolane as 5,800 mg/kg. A limit test conducted by Hoechst[19], reported the oral LD_{50} to be greater than 2000 mg/kg. In a 1949 report, Smyth, Carpenter and Weil reported the oral LD_{50} of Dioxolane as 3000 mg/kg.[20]

Dermal Route

In a journal article by Czajkowska et al., the dermal LD_{50} of Dioxolane was reported to be about 15,000 mg/kg in albino rabbits.[18]

Inhalation Route
Acute inhalation studies revealed that the 4-hour acute LC_{50} is 68.4 mg/l in Sprague-Dawley rats. Nominal exposure concentrations for were 201.9, 88.4, 67.9, 60.6 and 37.9, milligrams per liter (mg/l). Respiratory and neuromuscular abnormalities were the immediate responses to the test material during the exposure and during the four hour-post-exposure observation periods. Severity and incidences of these findings followed a concentration-related pattern. During the 14-day post-exposure observation period, all survivors appeared to recover by day 4. Most surviving animals appeared to regain pre-exposure body weights by Day 7 and showed normal weight gain patterns during the second week. Necropsy findings revealed high incidences of lung and liver discoloration as well as bladders distended with fluid and gastrointestinal tracts distended with gas in animals dying prior to scheduled sacrifice. The frequency of these findings appeared to be concentration related. [21]

Czajkowska, et al, also reported acute inhalation study results.[18] The LC_{50} concentrations were 118 mg/l in the rabbit (exposure time not given), 87 mg/l/ (4 hours) in the male rat and 166 mg/l (4 hours) in the guinea pig.

Summary: The acute toxicity of Dioxolane is well characterized by the available studies. There is a good correlation between the oral LD_{50} and the inhalation LD_{50}. Using a typical value of 75 ml for the minute volume of a 250-gram rat, the inhalation LD_{50} corresponds to a rat breathing in about 4900 mg/kg during the 4-hour inhalation exposure; this is similar to the oral dose producing lethality. Deaths occurred rapidly suggesting a CNS/respiratory system depression as the likely mechanism. Surviving animals showed normal weight gains within a few days after exposure.

Recommendation: Acute toxicity is sufficiently well characterized by the available studies. No additional testing is recommended.

Repeated Exposure Toxicity

Oral
In a 14-day gavage study conducted by Hoechst Celanese Corporation[22], groups of 10 rats of each sex were administered Dioxolane in corn oil by gavage for 14 consecutive days. Dosage levels were 0, 75, 250, 750 and 2000 mg/kg/day, plus a water-gavage control. Animals were sacrificed on the 15th day and necropsied. Histopathologic examination was conducted on several organs. Blood was removed for hematology. Chemically related mortality was limited to 3 high-dose males and 4 high-dose females during the study. Male rats at dosages of 250, 750 and 2000 mg/kg/day showed dosage-dependent decreases in platelet counts and reduced body weight gains and feed consumption values. The 750 and 2000 mg/kg/day dose groups had increased relative weights of the liver and lungs and reduced relative weight of the thymus. The 2000 mg/kg/day dosage group had increased relative kidney weights and reduced relative spleen weight. The 2000 mg/kg/day dosage of Dioxolane also produced adverse clinical signs, necropsy findings and histopathologic changes in the liver, thymus, kidneys and testes (centrilobular hepatocellular

hypertrophy and midzonal hepatocellular vacuolation; thymic atrophy; renal cortical tubular basophilia and dilatation and accumulations of birefringent intratubular crystals; subacute renal pyelitis and multifocal testicular degeneration (testicular degeneration in two of ten males).

In female rats, administration of Dioxolane at dosages of 750 and 2000 mg/kg/day caused increases in relative liver weights and a decrease in relative weights of spleen and thymus. The 2000 mg/kg/day dosage group also had increased relative kidney weights and a reduced relative pancreas weight. Dosages of 250 mg/kg/day and higher reduced body weight gains, feed consumption values and lymphocyte counts. Dosages of 750 and 2000 mg/ kg/day caused clinical observations, and the 2000 mg/kg/day dosage also caused a gross lesion, reduced platelet count and histopathologic changes in the liver, kidneys and thymus (centrilobular hepatocellular hypertrophy and midzonal hepatocellular vacuolation; thymic atrophy; subacute renal pyelitis). The NOEL for Dioxolane was 75 mg/kg/day.

In a 4-week "pilot study", groups of 20 male and 20 female albino rats, albino mice and Syrian hamsters were exposed to Dioxolane in drinking water at concentrations of 0, 0.5, 1.0 and 2.0%.[23] Body weight and water consumption were the only parameters recorded. Rats of each sex showed reduced body weight gains at the two highest dose levels. Body weight gains of high-dose mice and hamsters also appear to be affected but did not achieve statistical significance. The authors of the report only noted the statistically significant changes in body weight gains for rats (males, 1.0% and 2.0 % dose levels; females 2.0% dose level). Hamsters appear to be less sensitive to the toxic effects of dioxolane than rats and mice; rats appear to be the most sensitive based solely on body weight gains. The actual dosing for the male rats at 1% in drinking water with water consumption at 30 ml per day calculates to approximately 1000 mg/kg/day Dioxolane. This is the approximate dose level were body weight changes are observed in other repeat-dose studies.

Inhalation

A two week inhalation toxicity study, sponsored by Celanese was performed using groups of rats (5/sex/group) exposed for six hours per day, five days per week, for two weeks to Dioxolane vapor at concentrations of 0, 3 and 10 mg/l (984 and 3280 ppm). All animals survived the duration of the study. No treatment related observations were recorded. Body weights, organ weights, clinical chemistry parameters, and macropathology were unremarkable in treated animals. Depressed leukocyte values in male and female exposed animals may have indicated a response to treatment.[24]

In a 2-week inhalation study sponsored by Dow Chemical Co, groups of five male and five female Fischer 344 rats were exposed to concentrations of Dioxolane targeted to be 0, 500, 2000, and 5000 ppm. Animals were exposed for 6 hours per day, five days/week for nine exposures at measured concentrations of 0, 516, 2319, and 5132 ppm. Study parameters included body weights, organ weights, hematology, clinical chemistry, and gross and microscopic pathology. Exposure related and concentration-dependent effects were slight incoordination (5000 ppm males and females) during and following each exposure, decreased mean body weight during week two of exposure (5000 ppm males and females), and decreased white blood cell (WBC) counts (2000 and 5000 ppm males and females). Transitory incoordination was not associated with microscopic changes in tissues examined from the central and peripheral nervous system. Decreased WBC counts were not associated with morphologic alterations in the bone marrow, spleen, thymus, or lymph nodes. In addition, there were no exposure-related microscopic changes in any other organ or tissue. The NOEL for male and female rats was 500 ppm.[25]

Summary: The repeated-dose toxicity studies indicate that Dioxolane has a low order of toxicity with the most sensitive organ system probably being the blood-forming organs. There is a good correlation of effects and dose levels between the gavage and the inhalation studies. The NOAEL and LOAL for gavage were 75 and 250 mg/kg/day, respectively; and the NOAEL and LOAL of the 14-Day Dow inhalation studies were 500 and 2000 ppm, respectively. Using a 75 ml minute volume for a 250-gram rat assuming 50% absorption the 500 and 2000-ppm concentration correspond to about 80 and 325 mg/kg/day absorbed Dioxolane, respectively. Toxic effects and relative sensitivities, thus, appear not to be greatly route dependent.

Recommendation: The effects of repeated-dose administration of Dioxolane are sufficiently well characterized. No new studies are required

Subchronic Toxicity

A 13-week inhalation study in rats conducted by Dow Chemical Company (1990) used 10 rats per group and satellite groups of 10 rats of each sex per dose level and control that were allowed an 8-week recovery period. Dose levels were 0, 300, 1000 and 3000 ppm with a six-hour per day exposure five days a week. At the end of the exposure period, urine was collected, animals were sacrificed, blood was taken for hematology and chemical chemistry, and animals were submitted to a complete necropsy. Several organs were weighed and many tissues were fixed, sectioned, stained and examined microscopically. The satellite animals had blood samples taken at four and thirteen weeks of exposure and again at four and eight weeks into the recovery period. Satellite rats were necropsied at the end of the eight-week recovery period. In males, no significant effects were reported in the 300 or 1000 ppm group and the 3000 ppm group showed decreased alertness at the end of each exposure, reduction in leukocyte count, decreased urine specific gravity, decreased spleen weights and microscopic examination revealed that hepatocytes in the centrilobular regions of lobules were slightly larger and had more cytoplasmic eosinophilia than controls. In females, no significant effects were reported in the 300-ppm group. At 1000 ppm the females showed reduced spleen weights and increased relative liver weights. The 3000-ppm group showed decreased alertness at the end of each exposure, reduction in leukocyte count, decreased urine specific gravity and decreased spleen weights. Overall, the primary effect of dioxolane inhalation exposure to rats appears to be a reduction in white blood cells counts at 1000 or 3000 ppm. Rats appeared healthy, body weight gain was not affected and there was no corresponding myeloid toxicity, although there was a slight reduction in myeloid cells of the bone marrow at 3000 ppm. Exposure related pathologic effects were limited to slight enlargement of centrilobular hepatocytes of males at 3000 ppm. There was no indication of testicular toxicity based on careful light microscopic examination. The NOAEL was 1000 ppm for males and 300 ppm for females.[26]

Czajkowska et al. reported a seven-month gavage study. In this study, dioxolane was administered as a 20% aqueous solution to groups of 8-10 rats by gavage at 1/10 (580 mg/kg), 1/40 (145 mg/kg) and 1/80 (72.5 mg/kg) of the single-dose LD_{50} for 7 months. In addition, undiluted dioxolane was administered at 580 mg/kg to a group of rats for a period of 4 months. Mortality, appearance and behavior were recorded and body weight was determined once a month. After completion of the 7-month exposure period, some hematology and biochemistry parameters were measured, animals were necropsied and selected organ weights were determined. Effects were seen primarily in high-dose animals dosed with undiluted Dioxolane. These animals, dosed for only four months, showed reduced (about 20%) body weight gain, hypokinesis, slightly weakened muscular force and slight paresis of hind legs. The animals receiving the same dose level of Dioxolane as a water solution for seven months showed a slight increase in body weight gain. These high dose animals given aqueous dioxolane showed slightly reduced (20% as compared to control) activity of whole blood acetylcholinesterase. The 0.145 mg/kg dose group showed a 12% increase in activity of whole blood acetylcholinesterase. It was concluded that there was no evidence for “accumulative effects” of dioxolane under these conditions.[27] It is not known why the effects of undiluted dioxolane were different from diluted dioxolane at the same dose on a mg/kg basis. The aqueous high-dose of 580 mg/kg/day appears to be a NOEL with regard to body weight gain which was the best-documented adverse effect.

Summary: Subchronic administration of Dioxolane produced effects similar to those reported in the repeated-dose studies. The most sensitive target organ in the subchronic inhalation study was the blood forming system, manifest as reduction in WBCs and platelets and changes in spleen weight appear in rats. The inhalation NOEL for these effects was found to be 300 ppm for females and 1000 ppm for males in a 13-week study. The seven-month gavage study indicated low toxicity for the test material. The potential for neurotoxicity, suggested by the perceived neuromuscular effects at the high-dose level, was investigated by measurement of blood cholinesterase activity at the end of the exposure period. The statistically significant reduction in cholinesterase at the high dose was not supported by the lower dose, which showed higher activity than control. In addition, the correlation between blood cholinesterase and acetylcholinesterase in the neuronal receptor is weak and the magnitude of the observed depression is not convincing, especially in light of the length of exposure.[28] No other indication of neurotoxicity, other than transient solvent depression of the CNS, has been reported for Dioxolane and there is insufficient evidence to suggest neurotoxic potential based on these data.

Recommendation: The subchronic effects of Dioxolane are sufficiently well characterized for purposes of hazard and risk assessment. No new studies are recommended.

Genetic Toxicology *In Vitro*

The mutagenicity of Dioxolane was evaluated in Salmonella tester strains TA98, TA100, TA1535, TA1537 and TA1538 (Ames Test), both in the presence and absence of added metabolic activation, using Aroclor-induced rat liver S9 fraction, in a study sponsored by Celanese and conducted by Litton Bionetics. Dose levels were selected based on a preliminary toxicity determination. Dioxolane, diluted in ethanol, was tested at concentrations up to 50 µl/plate using the plate incorporation technique. Dioxolane did not cause a positive response in any tester strain with or without metabolic activation.[29]

An Ames test of Dioxolane was conducted by Goodyear in Salmonella tester strains TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of added metabolic activation by Aroclor-induced rat liver S9 fraction. Dioxolane, diluted in DMSO, was tested at concentrations up to 1000 µg/plate using the plate incorporation technique. Dioxolane did not produce a reproducible positive response in any tester strain with or without metabolic activation.[30]

Another Ames test of Dioxolane was conducted at Hill Top Labs using Salmonella tester strains TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of added metabolic activation by Aroclor-induced rat-liver S9 fraction. Dioxolane was tested using the plate incorporation technique at concentrations of 0.05 to 50 microliters per plate. Dioxolane did not demonstrate a positive mutagenic response under these conditions.[31]

The mutagenicity of Dioxolane was evaluated in *Salmonella* tester strains TA1535, TA1537 and TA1538 (Ames Test), and in *Saccharomyces cerevisiae* strain D4, both in the presence and absence of added metabolic activation by induced rat, mouse or monkey tissue S9 fraction from liver, lung and testis from animals pretreated with a "mixture of polychlorinated biphenyls". Salmonella were incubated with test substance both as suspension incorporation tests (at 0.75 and 1.5%) and as a plate incorporation test at 1.5% using rat liver lung and testis. With the tissues from other species, only plate-incorporation techniques were used for bacteria. Suspension tests with yeast were conducted at concentrations up to 5.0% using non-activation and activation conditions. Activation conditions for yeast cultures used only rat tissues (livers, lungs and testes). Dioxolane did not cause a positive response in any tester strain with or without metabolic activation.[32]

The ability of Dioxolane to cause aberrations in Chinese hamster ovary (CHO) cells in vitro was evaluated in the presence and absence of Aroclor-induced rat-liver S9 metabolic activation. Based on preliminary toxicity tests, nonactivated and activated cultures were treated with 0, 2.0, 3.0, 4.0 or 5.0 mg/ml. Nonactivated cultures were incubated with Dioxolane for 7.5 hrs and activated cultures were incubated for 2 hrs. A total of 100 cells were scored/each of two replicate cultures/dose level. There was no toxicity observed at the highest dose level in either the nonactivated or activated cultures. None of the test cultures exhibited a significant increase in the frequency of cells with chromosome aberrations in either the nonactivated or activated systems compared to the negative control.[33]

The ability of Dioxolane to induce specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells (Mouse Lymphoma Mutagenicity Assay) was evaluated in the presence and absence of Aroclor-induced rat liver S9 metabolic activation. Based on preliminary toxicity determinations, nonactivated cultures were tested with concentrations ranging from 750 nl/ml to 5000 nl/ml, producing a range of 85 - 61% relative growth. Activated cultures treated with concentrations ranging from 750nl/ml to 5000nl/ml produced a range of 77.8 - 95.1% relative growth. None of the cultures produced mutant frequencies significantly greater than the water control.[34]

Dioxolane was tested in the presence and absence of an activation system for detection of its DNA damaging potential in the SOS assay. This assay is based on the test agent inducing beta-galactosidase (GAL) activity in Salmonella-typhimurium strain (TA-1535/pSK1002). GAL is coded by the lacZ gene and controlled by the fused umuC and lacZ gene in the pSK plasmid.by induction of umu gene expression. The potential to induce SOS response was monitored by measuring the cellular 6-galactosidase activity. Under the conditions of this assay, Dioxolane was negative.[35]

Dioxolane was tested in C3H/101 1/2 cell cultures in vitro for cytotoxicity and cell transformation. Evidence of transformation was observed in the colony and foci assay methods. Because of the dose range employed and the type of transformation observed, Dioxolane was considered to have weak positive activity in C3H/101 1/2 cell cultures.[36] The data suggest transforming activity but there are serious issues of concern with the data. The poor reproducibility on different days, the lack of clear dose-concentration relationships, the test material sporadically giving results similar to positive controls and at other times no response, and the lack of high-concentration toxicity data makes it difficult to conclude that this assay represents a positive finding. A more appropriate interpretation of the data is “not interpretable” as to genotoxic potential.

Dioxolane was tested for its ability to produce cell transformation in Balb/c-3T3 cells. Treatments were conducted at multiple dose levels, including slightly cytotoxic concentrations, in sealed flasks to prevent volatilization. . The transforming activities of all five doses of Dioxolane were comparable to the spontaneous background average of 0.12 foci/culture vessel and there was no evidence of a dose-related response with the test material. This range of test material treatments corresponded to 0 to 85% survival in the simultaneous colony survival assay and did not induce a significant increase in the appearance of transformed foci. Therefore, the test material was considered to be inactive in the Balb/c-3T3 in vitro transformation assay.[37]

Genetic Toxicology *In Vivo*

The possible genotoxic effects of dioxolane were studied using the micronucleus test in a published study. Mice were given dioxolane ip in two doses at 24 hr intervals. Dioxolane at doses from 1500 mg/kg to 6000 mg/kg caused a significant increase of micronucleated polychromatic erythrocytes in bone marrow of mice as compared to the negative control value. Positive results in the micronucleus test provide evidence of genotoxic activity for Dioxolane. This study was considered potentially spurious, as no other genotoxic activity of Dioxolane was known, the study was not conducted under glp conditions, experimental details were lacking and the purity of the test material was not specified.[38] Because of the questions surrounding the reliability of this report, Hoechst Celanese conducted a more robust glp-study in which no genotoxic activity was found. This is reported below.

A glp mouse micronucleus test was sponsored by Hoechst Celanese Corporation in which groups of five ICR mice of each sex received Dioxolane in corn oil by i.p. injection at 0, 1576, 2048, 2663, 3462 or 4500 mg/kq. Mortality at the end of a seven-day observation period was used to calculate an i.p. LD_{50} of 2603 mg/kg. The high dose for the micronucleus study was set at 80% of the LD_{50}, which was 2100 mg/kg. Lower levels were 1050 and 525 mg/kg. Groups of mice of each sex were injected with these three dose levels of Dioxolane and sacrificed in groups of five at 24, 48 and 72 hours post injection. Excessive mortality occurred at the high dose level and additional animals were injected to allow sufficient high-dose animals for sacrifice at 72 hours post injection. The number of micronucleated cells was not increased by treatment with Dioxolane while the number in the positive control was significantly increased and within the expected range. The high mortality observed in the high-dose-group and significant bone marrow toxicity at the high dose level indicated that the test material was administered at the highest practical level. Under the conditions of this assay, Dioxolane did not show genotoxic activity.[39]

Dioxolane was tested for induction of single-strand breaks of DNA in rat hepatocytes in vivo. Male rats were injected intraperitoneally 4 hours before decapitation with dioxolane at a single dose of 290 mg/kg or 580 mg/kg. Single-strand breaks in DNA were detected by the alkaline elution technique. The elution rates were significantly higher than these of DNA from control rat hepatocytes, but no dose effect relationship was found. Under the conditions of this assay, dioxolane was considered to induce single-strand breaks in DNA.[40] These results are possibly spurious and related to an impurity of the test material which may have been derived from the same source as the test material used in the reported-positive micronucleus test.

An oral evaluation of the ability of Dioxolane to increase the frequency of dominant lethal mutations in the germ cells of male rats was conducted. Male rats that received Dioxolane daily for eight weeks were evaluated for effects on fertility in a weekly mating design. Oral doses of 0, 580 and 1160 mg/kg/day were administered five days per week for eight weeks. Every week during the treatment period, each male was mated with two females. This dosing regime had no effect on male fertility indexes. Pregnancy outcomes were also comparable to controls. System toxicity was apparent as body weight gains in the 580 mg/kg dose level were 47% of control and body weight gain in the 1160 mg/kg dose group was only 22% of control. Liver kidney and spermatic vesicle weights were increased in dosed animals. Although no functional deficit in fertility was recognized in these males, microscopic evidence of testicular degeneration and alterations in spermatogenesis were noted. Three of nine high dose males showed atrophy, necrosis and seminiferous epithelium exfoliation and three low-dose males were reported to show seminiferous epithelium exfoliation (one animal showed the presence of large multinucleated cells). There was no evidence of a dominant lethal effect.[41]

A 12-month inhalation study was conducted using male rats to evaluate the ability of Dioxolane to increase the frequency of dominant lethal mutations in germ cells. Male rats were exposed to Dioxolane five hours daily, five days a week, for twelve months, at a concentration of 2500 mg/m3. At the end of the 12-month exposure period, males (14 per group) were mated through one week with female rats in a ratio of 1:2. Dams were sacrificed 13-14 days after the middle of mating intervals for evaluation of conception products. No increase in the number of preimplantation losses, dead implants and live fetuses per female was noted in any treated group as compared to the appropriate control group. Dioxolane did not affect the fertility of males; however, histopathological changes in the testis were reported. The authors reported that the frequency of necrosis in the seminiferous epithelium was much higher in treated animals than in controls but details were not provided. No compound induced dominant lethal mutations in germ cells were found to result from inhalation exposure to Dioxolane under these conditions.[42]

Summary: Dioxolane has been extensively tested in genotoxicity assays. The weight of the evidence clearly indicates lack of significant genotoxic potential for this material. In addition, the few studies which suggest genotoxicity carry lower reliabilities.

Recommendation: Dioxolane has been sufficiently characterized for genotoxic activity. No new studies are recommended.

Reproductive Toxicology

In a one-generation reproduction study, male Charles River rats (5/group) were exposed to Dioxolane in their drinking water at concentrations of 0, 0.5 or 1.0% for 90 days prior to mating with previously untreated females (10/group, two females per male). Treatment continued through the mating period. Dams and pups were examined with respect to survival and body weight through postpartum day (PD) 21 and the animals were retained for further experimentation. Significant differences were observed between treated and control animals in the following: decreased maternal body weights (high-dose group on PD 1 and 4, decreased rates of coupling and parturition, and increased number of stillborn pups (both treatment levels), decreased survival of pups in both treatment groups, and decreased numbers of pups in the high-dose group. No significant differences were observed between treated and control animals in the following: parental mortality, fecundity or female fertility indices, gross external abnormalities, and pup body weights.[43] In the second phase of the study, untreated proven-breeder male rats were mated with the dams that produced the F1a litter. The dams were treated with test material continually from the initial (F1a) mating throughout gestation, lactation and a 10-day rest period after weaning of F1a litter before the second mating period. Neither dams nor males were exposed after the F1b mating interval started. Dams and F1b pups were examined with respect to survival and body weight through postpartum day 21, the F1b pups were retained for further testing, and the dams were sacrificed and subjected to histopathological examination. Significant differences observed between treated and control animals in the following: decreased fecundity index, incidence of parturition, and female fertility index at both treatment levels. No significant differences were observed between treated and control animals in parental mortality, male fertility index, number of pups born, stillborn, cannibalized, or viable pups (at birth or on lactation days 1, 4, 12 and 21), maternal or pup body weights, gross maternal pathologic examination, weights maternal organs (adrenals, both gonads, liver, pituitary, and uterus), and maternal histopathologic examination.[44]

In a one-generation reproduction study, male Charles River rats (5/group) were orally exposed to dioxolane in their drinking water at concentrations of 0, 0.01, 0.03 or 0.10% for 90 days prior to mating with previously untreated females (10/group, 2 females/male). Treatment continued through mating, gestation and lactation. Dams and pups were examined with respect to survival and body weight through postpartum day (PD) 21 and the animals were retained for further experimentation. A significant, but likely spurious, difference was observed between treated and control animals in male pup body weight (decreased, low-dose group on lactation day 21). No significant differences were observed between treated and control animals in the following: maternal mortality, mating and fertility indices, male and female fertility, incidence of parturition, gross abnormalities in pups, number of pups delivered stillborn or viable, or cannibalized (lactation days 1, 4, 12 or 21), survival indices, female pup body weight, and female parental body weight.[45] The high dose, 0.10% in drinking water, is considered a LOAEL.

In a single-generation reproduction study, male Charles River COBS albino rats (5/group) were exposed by inhalation to Dioxolane at nominal (measured concentrations were close to nominal values) concentrations of 0 or 125 ppm for seven hours a day, five days a week, for 90 days prior to mating with untreated females (10/group, 2/male). Each sex was treated during mating and females were treated until 1-2 days before delivery. Females and F1a pups were retained for further studies, males were sacrificed and necropsied after mating. A significant difference was observed between treated and control animals with respect to decreased relative paternal lung weight for the F1a male parental generation. No significant differences were observed between treated and control animals in the following: mating, fecundity, male female fertility indices, incidence of parturition, gross external pup abnormalities, number of pups delivered, stillborn, cannibalized or viable pups and number of viable pups through 21 days, pup survival and body weights, maternal body weights, gross paternal necropsy examinations.[46] In the second part of this study, after weaning the F1a litter, dams were mated with new males that had been exposed to Dioxolane for 120 days prior to mating. Rats of each sex were exposed to Dioxolane vapors during the 15-day mating period and the pregnant females were exposed until 1-2 days before delivery. No significant differences were observed between treated and control animals in fecundity or male fertility indices, ratio of parturition/pregnancy, gross external abnormalities in pups, number of pups delivered, delivered stillborn, cannibalized or viable, or number viable pups or mean pup weight through 21 days, F1b pups survival data, and maternal body weight. The female mating index was reduced but not with statistical significance.[47] In summary, although treatment did not significantly affect any measured reproductive parameter, there was a tendency toward an overall reduction in the number of pups born and weaned in both the F1a and F1b litters of the dosed group. The lack of statistical significance may partly be a function of the low numbers of animals employed in the study. The possibility that the 125-ppm exposure represents a LOAEL for reproduction in the F1a and F1b litters cannot be excluded.

Effects on male fertility and the frequency of dominant lethal mutation in germ cells of male rats were studies by exposing male rats to 2500 mg/m^3 (830 ppm) Dioxolane, five hours a day, five days a week for 12 months or by exposing male rats to oral gavage administration of Dioxolane for eight weeks at 580 or 1160 mg/kg/day (5 days per week for 8 weeks). Exposed rats were mated with unexposed females. Male fertility was unaffected by these dosing regimes as were other parameters indicative of a dominant lethal effect. Some treated males were reported to display focal necrosis of the seminiferous tubules. These studies are more fully described in the genotoxicity section of this document.[42]

Discussion of Reproductive Toxicology

Adverse reproductive effects of Dioxolane were reported at high dose levels in the drinking water reproduction (one-generation) studies. Most notable effects were the apparent reduced female mating index, live birth index and pup survival index at the high dose level (1.0% in drinking water) for the F1a litter in which the females were exposed during mating and gestation. Examination of the data suggests that effects occurred at the 0.5% drinking water level and were more severe at the higher dose. The other drinking water study, at lower concentrations, was below the NOAEL. It could not be clearly determined which was the affected sex due to the study design. The LOAEL for reproductive effects may also be an effect level for maternal toxicity. There was clear maternal toxicity at the 1.0% level and mild effects on body weight gain at the 0.5% level. This same strain of rats was also examined in a 4-week "pilot" study where only body-weight gains and water consumption was recorded.[23] In this study, body weight gain was reduced over the 4-weeks of the study for female rats by 15, 28 and 56 percent at 0.5, 1.0 and 2.0 percent drinking water levels, respectively. Although the reduced body weight gain was not statistically significant at the two lower doses (due to an n of only 5), it appears that 0.5% represents a maternally toxic concentration in drinking water.

Adverse effects on reproduction thus are considered to occur at maternally toxic doses. After consideration of the data and the information that higher gavage or inhalation doses do not cause dominant lethal effects and the additional data from the Sitarek et al study[49], it can be concluded that the affected sex in this study was probably the female. The perinatal mortality reported is supported by the developmental toxicity study of Sitarek el al.[49] (described in detail under developmental toxicology) in which increased perinatal mortality was reported after dosing pregnant dams every-other-day at 1150 mg/kg Dioxolane.

Another consideration in assessing the potential reproductive toxicity of Dioxolane is histopathologic changes in the reproductive organs. Three repeat-dose or subchronic glp-studies conducted careful examinations of male and female reproductive organs from exposed rats and in only one study was there any finding associated with reproductive organs. In the 14-day gavage study at doses up to 2000 mg/kg/day[22], two of ten high-dose males displayed multifocal testicular degeneration which was described by the pathologist as “the affected tubules had a decreased amount of spermatogenic cells with evidence of degeneration and formation of multinucleated giant cells” Other males at this dose-level were not affected or were females (regarding their reproductive organs) or any lower-dose animals. In the 14-day inhalation study at concentrations up to 5132 ppm[25] (inhalation of approximately 1700 mg/kg over 6 hours) no male or female demonstrated reproductive organ effects. And, in the 90-day inhalation study at concentrations up to 3010 ppm[26] (inhalation of approximately 1000 mg/kg over 6 hours), neither males nor females were found with adverse changes to reproductive organs. Taken together, this is strong evidence that Dioxolane is not a significant reproductive toxicant.

Summary: Dioxolane has an effect on reproductive parameters only at maternally toxic doses. The clear NOAEL for these effects was 0.1% in drinking water.

Recommendation: Reproductive effects of Dioxolane are characterized sufficiently for the purpose of hazard and risk assessment. No additional studies are recommended.

Developmental Toxicology

In a developmental toxicity study sponsored by Hoechst Celanese in 1991, groups of 25 presumed-pregnant rats received Dioxolane in corn oil by gavage from day 6 to 15 of gestation. Dosage levels were 0, 125, 250, 500 and 1000 mg/kg/day. Animals were sacrificed on gestation-day 20, opened and examined for pregnancy, number and placement of implantation sites, early and late resorption, live and dead fetuses and number of corpora lutea. Fetuses were subsequently examined for gross external, soft tissue and skeletal alterations. No rats died during the study and adverse clinical signs were not reported at any dose level. Body weights of dams were reduced between gestation day (gd) six and seven for 500 and 1000 mg/kg animals. Transient reductions in feed consumption occurred for the 500 and 1000 mg/kg dose groups. Data from the preliminary range-finding study also indicate that dose levels of 250 mg/kg and above are associated with adverse effects related to the toxicity of the test material. The 1000 mg/kg dosage level was associated with significantly reduced fetal body weights and significantly increased litter and fetal incidences of externally evident tail malformations, vertebral malformations interrelated with the tail malformations and septal effects in the heart. High dose fetuses also displayed delayed ossification and one fetus was found with a cleft palate. Significant adverse fetal effects were not found in the 500-mg/kg group. The NOEL level for dams was considered to be 250 mg/kg and the developmental NOEL was considered to be 500 mg/kg. Therefore, Dioxolane is not considered a specific developmental toxicant under the conditions of this study.[48]

A 1992 publication authored by Sitarek et al.[49] describes a developmental toxicity study of Dioxolane. The study was divided into two parts: prenatal and postnatal development. In the first part, female rats were given by gavage every other day from days 8-20 of gestation an aqueous solution of dioxolane at daily doses equal to 0.025, 0.1 and 0.2 LD_{50} (0.14, 0.58 or 1.15 g/kd/). In the second part (perinatal toxicity), Dioxolane was administered every other day from days 2-20 of gestation at daily doses equal to 0.025, 0.075 and 0.15 LD_{50} (as stated in the material and methods section, dose levels reported in the results and discussion section are the same as in the first experiment and it cannot be determined what doses were actually used in the second part). Dioxolane did not cause increased embryo or fetus intrauterine death rates or congenital defects at any dose level. In the pre-natal study, the mid (0.58 g/kg) and high dose (1.15 g/kg) were reported to be associated with dose-related delays in fetal development. High-dose pups also weighed less and showed reduced mean crown-rump lengths. Examination of the presented data suggests that the mid-dose effect is marginal and probably represents a fetal NOEL of 0.58 g/kg. Maternal toxicity was manifest at the high dose by reduction in body weight gain and an increase in relative adrenal weights at sacrifice the maternal NOEL appears to be 0.58 g/kg.

In the perinatal study, Dioxolane did not cause impairment of physical development or behavioral disturbances. Exposure to higher doses of the compound (stated as 1.15 g/kg) leads to increased perinatal death rates in the offspring, without causing disturbances in the maternal instinct. The exposure of pregnant rats to dioxolane decreased hemoglobin levels in 5-week-old offspring but the levels had returned to control by 8 weeks. At a dose 1.15 g/kg (0.2 LD_{50}) the chemical significantly increased exploratory motor activity of female offspring at the age of 8 weeks, but did not affect significantly locomotor activity of males and the active avoidance acquisition of adult offspring. High-dose males were reported to have reduced body-weight gain between the 14th and 15th week after birth. Perinatal deaths were also increased it the high-dose group.

These two developmental toxicity studies used an unusual (every other day) dosing schedule that might be expected to increase developmental toxicity relative to maternal toxicity. This makes it more difficult to compare these results to others. The lack of significant malformations, and the apparent equivalence of the maternal and the developmental NOELs suggest low potential for developmental toxicity from Dioxolane exposure.

Summary: the pre-natal developmental toxicity of Dioxolane had been well characterized by a 4-dose gavage study demonstrating that Dioxolane is not a specific developmental toxin. At maternally toxic doses, administration is associated with increased malformations and delayed ossification. The study by Sitarek is supporting in that doses of 575 or 1150 mg/kg/day were not found to cause adverse effects on the conceptus other than developmental delays. It is not known, however, why malformations were not reported at these maternally toxic doses since they were reported in the corn-oil gavage (Argus) study. The dosing vehicle in the Argus study was corn oil while the Sitarek study used water; differences in pharmacokinetics based on absorption rates are a possible explanation. Nevertheless, the developmental toxicity of Dioxolane appears low.

Recommendation: No new studies are recommended as this endpoint is sufficiently well characterized.

Other Studies (Non-HPV)

Absorption/Distribution/Metabolism/Excretion
Snipes et al. published a paper entitled *A method for measuring nasal and lung uptake of inhaled vapor* in which Dioxolane was used a model compound for demonstrating methodology. This paper describes apparatus and methods for measuring uptake of inhaled vapors in the nose and lungs of dogs. The system allows sampling of air from the trachea at specific times during inspiration and expiration without surgical manipulation of the animal, thus allowing repeated studies in the same animal. During exposure, the dogs are anesthetized and cyclic respiratory patterns are maintained by means of an external respirator. A pneumotachograph installed in the exposure line is connected to a respiratory monitoring system that both monitors the dog's respiratory pattern and triggers sampling at specific times in the respiratory cycle. Air sampling, both at the nose and within the trachea, can be done during the entire breathing cycle or during specific portions of it. Vapors are sampled at a point just external to the dog's nose and from within the trachea through a modified endotracheal tube. To develop and demonstrate the system, three beagle dogs were exposed to Dioxolane at nominal vapor concentrations of 500 ppm; vapor sampling was triggered for the entire inspiratory and expiratory portions of the breathing cycle during 10-min exposures. After correcting data to account for vapor that desorbed from the nasal passages during exhalation (after initially being absorbed in the nose during inhalation), net nasal uptake of Dioxolane was 66.6%. Lung uptake was 2.1% for Dioxolane.[50]

It can be concluded from this study that upper respiratory tract uptake of Dioxolane is an important absorption mechanism for dogs under these experimental conditions. Upper respiratory tract absorption is likely to also be an important uptake mechanism in humans.

Carcinogenicity
No known completed and reported studies of the carcinogenic potential of Dioxolane are available; however, the literature indicates that at least two had been started.

PPG Corporation sponsored a chronic (2-year) drinking water study at Industrial Bio-Test Laboratories. The in-life portion of the study was started in April 1975 and a draft report dated 7/29/1985 was audited against laboratory data by Experimental Pathology Laboratories (EPL). In the August 1985 audit report from EPL, it was concluded that the study contains accurate toxicological information concerning the effects of dioxolane administered to the drinking water of male and female rats. The study had concluded that there were no statistically significant treatment-related effects in body weight or food and water consumption. A slight reduction in testicular weights and an increase in spleen weights in treated male rats was not statistically-significant[51]. The final report of the chronic study was not found in the open literature and it is presumed that it was never issued.

There is reference to a chronic inhalation study in progress from which males were used as breeders for the one-generation inhalation reproduction study.46 It is not known if this chronic inhalation study was completed or reported.

Conclusions

Examination of the entire data set for Dioxolane indicates that there is a low priority for additional studies on this material. Dioxolane is mildly toxic by oral administration or by inhalation. After acute exposure to high levels, reversible CNS depression, typical of most volatile solvents, appears to be the primary effect. After repeated dose or subchronic administration, effects on the blood forming systems, manifest as reduction in WBCs and platelets and changes in spleen weight appear to be the most sensitive target organs in rats. The inhalation NOEL for these effects was found to be 300 ppm for females and 1000 ppm for males in a 13-week study. The oral gavage NOEL was found to be 75 mg/kg/day in a 14-day study. After oral administration to pregnant females, developmental delays and perinatal mortality are found only at maternally toxic doses. Reproductive effects are only apparent at maternally toxic levels. The weight of evidence indicates that Dioxolane has low genotoxic potential.

The current exposure pattern is restricted to industrial workers involved in the manufacture and subsequent use of Dioxolane. Monitoring data indicate that typical exposures are below 1.0 ppm, which is at least two orders of magnitude below the inhalation 13-week NOEL. It is unlikely that Dioxolane will have any adverse effect at this exposure level. Ferro and Ticona are aware of no consumer exposure to Dioxolane.

Studies on aquatic organisms indicate a low order of acute toxicity for Dioxolane to fish, invertebrates or aquatic plants. Volatility limits the concern for chronic effects in the aquatic environment and data from plant effluents indicate that the levels being released from manufacture are less than 0.1 ppm in plant outfalls.

This analysis shows that Dioxolane has a low potential for harming the either human health or the environment and it is concluded that additional studies would not add significant information. No additional studies are recommended to fill the requirements of the EPA HPV Challenge Program.

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Robust Summaries

Melting Point

Type	Melting Point
Test Substance	1,3-Dioxolane CAS Number: 646-06-0
Method	
• Guideline	None
• Test Type	Melting Point
• GLP	No
• Year	Unknown
Result	
• Melting Point	-95 deg C
Remarks Field for Results	Handbook data
Conclusions	
Remarks Field	The melting point is –95˚C
Data Quality	
• Reliability	Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.
References	1. Lide, D.R. (ed). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 1991-1992.,p. 3-218
Other	

Boiling Point

Type	Boiling Point
Test Substance	1,3-Dioxolane CAS Number: 646-06-0

Method

- Guideline: None
- Test Type: Boiling Point
- GLP: No
- Year: Unknown

Result

- Boiling Point: 78 deg C @ 765 mm Hg (1)

Remarks Field for Results: Handbook data

Conclusions

Remarks Field: Boiling point is 78 deg C @ 765 mm Hg (1)

Data Quality

- Reliability: Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.

References

1. Lide, D.R. (ed). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 1991-1992. p. 3-218

Other

Vapor Pressure

Type Vapor Pressure

Test Substance 1,3-Dioxolane
CAS Number: 646-06-0

Method

- Guideline None
- Test Type Vapor Pressure
- GLP No
- Year Unknown

Result

- Vapor Pressure 70 mm Hg @ 20 Deg C

Remarks Field for Results Handbook data

Conclusions

Remarks Field Vapor pressure is 70 mm Hg at 20˚C

Data Quality

- Reliability Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.

References Sax, N.I. and R.J. Lewis, Sr. (eds.). Hawley's Condensed Chemical Dictionary. 11th ed. New York: Van Nostrand Reinhold Co. p 425 (1987).

Other

Partition Coefficient, Octanol-Water

Type Partition Coefficient, Octanol-Water

Test Substance 1,3-Dioxolane

Method

- Guideline: Not specified
- Test Type: Partition Coefficient, Octanol-Water
- GLP: No
- Year: 1995

Result

- Log $k_{o/w}$: Experimental -0.37[1]; Calculated by KOWWIN -0.31[2]

Remarks Field for Results: Dioxolane was one of the reference compounds for development of the KOWWIN program (module of EPIWIN). The experimental value is from the literature. The calculated value is the result of the KOWWIN calculation. The experimental value is given priority and the calculated value is supporting.

Conclusions

Remarks Field: The log $K_{o/w}$ is approximately –0.35. This material is expected to be relatively water soluble and not bioaccumulate to any significant degree.

Data Quality

- Reliability: Klimisch Code 2. A reliability code of 2 is generally assigned to literature values not conducted under OECD guidelines or glps.

References

1. Hansch. C., A. Leo and D. Hoekman. Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. ACS Professional Reference Book. Wshington, DC: American Chemical Society. 1995.
2. KOWWIN v 1.66, Syracuse Research Corporation, Syracuse, NY (April 2000)

Other

Water Solubility

Type	Water Solubility
Test Substance	1,3-Dioxolane
Method	
• Guideline	None specified
• Test Type	Water Solubility
• GLP	No
• Year	Unknown
Result	
• Solubility	Soluble in water in all proportions.
Remarks Field for Results	Handbook data
Conclusions	
Remarks Field	Material is soluble in water in all proportions.
Data Quality	
• Reliability	Klimisch Code 2. A reliability code of 2 is assigned to data from reference handbooks.
References	1. Lide, D.R. (ed). CRC Handbook of Chemistry and Physics. 72nd ed. Boca Raton, FL: CRC Press, 1991-1992.,p. 3-218
Other	

Photodegradation

Type Photodegradation

Test Substance 1,3-Dioxolane

Method

- Guideline: Estimated Using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN)[1] which estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical.
- Test Type: Photodegradation Estimate
- GLP: No
- Year: 2000

Results

- Result: APOWIN estimated OH rate constant 11.2 x 10-12 cm3/molecule-sec

Remarks Field for Results: The APOWIN estimate for the reaction rate is based on simple hydrogen abstraction. Similar compounds provide estimates close to measured values for this rate constant. Thus, the method is expected to provide an accurate estimate of the reaction rate constant with hydroxyl radical. Based on the estimated rate constant and using the defaults in APOWIN for atmospheric hydroxyl radical concentration, the estimated half-life is approximately 11.5 hours.

Conclusions

Remarks Field: The atmospheric half-life of 1,3-Dioxolane in the atmosphere is estimated to be in the range of 11.5 hours

Data Quality

- Reliability: Klimisch Code 2. A reliability code of 2 is assigned a result using an accepted method of estimation.

References

1. Syracuse Research Corporation, Syracuse, NY (April 2000)

Other

Water Stability

Type Water Stability

Test Substance 1,3-Dioxolane
CAS Number: 646-06-0
Purity 99.98%

Method

- Guideline: OECD 111 (1981)
- Test Type: Hydrolysis as a Function of pH
- GLP: Yes
- Year: 2000

Remarks Field for Test Conditions

◊ Duration: Four days (preliminary test only)

◊ Analytical Method: Direct injection into GC using flame ionization detector.

◊ Buffers:

Target pH	Buffer System	Measured pH at 50˚ C
4.0	Potassium biphthalate	3.96
7.0	Potassium phosphate	7.03
9.0	Sodium borate	9.04

◊ Vessels: Amber Teflon-lined screw cap vials

◊ Replicates: Two at each pH.

◊ Temperature: 51 ± 0.2˚ C

◊ Additional Testing: Not conducted since material showed less than ten percent degradation at 50˚ in four days.

Results

- Nominal Target Concentration — 400 mg/l
- Measured Concentrations

pH	Measured Concentration (mg/l)		Percent Degradation At 51˚ C in 4 days
	Initial	Final	
3.96	484	574	2.1
7.03	345	342	0.9
9.04	529	523	1.2

- Percent Degradation — Less than 10% at 50˚ in four days
- Breakdown Products — None

Remarks Field for Results — Data were consistent at all pH values, there may have been slightly more degradation at the lower pH.

Conclusions

Remarks Field

- Dioxolane is stable at pH 4, 7 or 9 for four days at 51˚ C under the conditions specified in the OECD 111 Guideline.
- Dioxolane is considered to have a half-life ($t_{1/2}$) at 25 ˚ C greater than one year at pH 4, 7 and 9.

Data Quality

- Reliability — Klimisch Code 1. May be used without restriction.

Reference — 1,3-Dioxolane CAS No. 646-06-0: Hydrolysis as a Function of pH. Toxikon Corporation, Laboratory Project ID 00J0009f, October 2000.

Other — The hydrolysis-modeling program found in EPIWIN contains no valid model for estimating hydrolysis of ethers.

References for supporting studies

1. HYDROWIN modeling program, version 1.67, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Theoretical Distribution (Fugacity)

Type	Theoretical Distribution (Fugacity)
Test Substance	1,3-Dioxolane
Method	
• Guideline	Estimated using the MacKay model with standard defaults contained in EPIWIN v 3.05.[1]
• Test Type	Level III Fugacity Model
• GLP	No
• Year	2000
Result	
• Distribution	○ Air 4.1 % ○ Water 54 % ○ Soil 42 % ○ Sediment 0.1 %
Remarks Field for Results	This is the currently accepted model for theoretical distribution estimation.
Conclusions	
Remarks Field	This material is expected to environmentally distribute primarily in water and soil.
Data Quality	
• Reliability	Klimisch Code 2. A reliability code of 2 is assigned a result using an accepted method of estimation.
References	1. Syracuse Research Corporation, Syracuse, NY (April 2000)
Other	

Biodegradation

Type	**Ready Biodegradation**	
Test Substance	1,3-Dioxolane CAS Number: 646-06-0	
Method		
• Guideline	OECD 301D	
• Test Type	Ready Biodegradation	
• GLP	Yes	
• Year	2000	
• Contact Time	35 Days	
• Innoculum	Municipal Water Treatment Secondary Effluent	
Remarks for Test Conditions	◊ Innoculum	• Fresh domestic water treatment secondary effluent collected about one week prior to test • One ml filtered effluent per liter final concentration. • Not acclimated
	◊ Test Material Concentration	• 3.04 mg/l
	◊ Reference Material	• Sodium Benzoate at 3 mg/l • Reference material showed 78% of THOD
	◊ Incubation Temperature	• 22˚ C in the dark
	◊ Sampling Frequency	• 0, 3, 5, 11, 14, 17, 21, 28 and 35 days • Duplicate bottles sampled
	◊ Analytical Method	• Oxygen consumption
	◊ Controls and Blanks	• Innoculum blank without test material • Positive control using Sodium Benzoate

Result

• Degradation Percent after time	3.7 % Biodegradation in 35 days
• Result	Not Readily Biodegradable
• Kinetics	Not applicable
• Breakdown Products	None determined
Remarks Field for Results	Not readily biodegradable

Conclusions

Remarks Field — Not readily biodegradable under these conditions

Data Quality

- Reliability — Klimisch Code 1. Recent glp study meeting current OECD guideline, may be used without restriction

References — 1,3-Dioxolane (CAS 646-06-0): Ready Biodegradability: Closed Bottle Test. Toxikon Corporation September 2000, submitted to Ticona and Ferro Corporations

Other — This study is supported by an earlier study, sponsored by Celanese, in which Dioxolane was tested for biodegradation using a municipal secondary effluent and measuring oxygen consumption with a manometric respirometer. After 15 days Dioxolane showed 5.2 % of the THOD. [1]

This result is supported by the BIOWIN V4.0 model found in EPIWIN. Two of the three models included predict that Dioxolane will not rapidly biodegrade.[2]

References for Supporting Studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Acute Toxicity to Fish

Type	Acute Toxicity to Fish
Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Purity 99.98%
Method	
• Guideline	OECD 203 (1992)
• Test Type	Acute Toxicity to Fish
• GLP	Yes
• Year	2000
• Species/Strain	Bluegill sunfish (*Lepomis macrochirus*)
• Analytical Monitoring	At the beginning and end of each 24-hour renewal period
• Exposure Period (unit)	96 hours
• Test Details	Static renewal, renewal every 24 hour
• Statistical Methods	None applied to biology due to lack mortality

Remarks Field for Test Conditions	◊ Fish Size and Age	*Lepomis macrochirus* juveniles, 29 ± 1.9 mm average standard length and 0.57 ± 0.11 g average wet weight.
	◊ Test conditions	As specified in OECD 203
	◊ Solvent	None, test material water soluble
	◊ Dilution Water Source and Chemistry	Filtered city of Jupiter (Florida) freshwater with an initial hardness and alkalinity of 66 and 28 mg/l as $CaCO_3$, respectively and an initial dilution water temperature range of 23.1 to 23.2 °C. Final hardness and alkalinity on day 4 were 74 and 30 mg/l as $CaCO_3$, respectively.

◊	Stock and Test Solutions	A stock solution with target concentration of 10,000 mg/kg was prepared by weighing the test material and dissolving it in dilution water. This stock was diluted to prepare the test solutions each day. Concentration of Dioxolane in both the stock and the test dilutions were determined by gc analysis using a glp validated method.
◊	Vessels and Lighting	The test chambers were 3.5-gallon glass jars (22-cm diameter x 30-cm height) containing 9.0 L of dilution water and providing a water depth of approximately 25 cm. All test chambers were covered throughout the exposure period to reduce evaporation or contamination. The test chambers were positioned in a water bath set to maintain 23 ± 2ºC under fluorescent lighting regulated to a photoperiod of 16 hours light and 8 hours darkness. The light intensity ranged between 11.9 and 17.1 microMols per square meter per second
◊	Fish per Vessel and Group	There were 10 fish in each vessel, 30 fish (3 vessels) per treatment and control
◊	Dose Selection	Dose selection of 100 mg/l was based on a range-finding study under static conditions with concentrations as high as 1000 mg/l showing no mortality.
◊	Water Chemistry in Control and in One Concentration Where Effects Were Observed	Dissolved oxygen averaged about 8.5 mg/l at the beginning of each renewal period and was found to be between 5.9 and 6.9 mg/l at the end of each 24-hour period. Only one measurement of 48 total was below 6.0 mg/l. The initial pH varied from 7.7 to 8.0 at the beginning of each 24-hour renewal period and ranged from 6.9 to 7.7 at the end of each period (only one measurement of 48 total was below 7.0). The pH and DO were similar in the control and treatment group.
◊	Renewal	Solutions were renewed after 24 hours. The test material was known to be volatile.
◊	Exposure period and Observations	Four days with daily observations

◊ Temperature range: 22.0 to 23.2º C as measured at eh beginning and end of each 24-hour renewal. The diurnal range of the water bath temperature was continuously monitored using a minimum/maximum thermometer and recorded daily and ranged from 21.6 to 23.4º C over the testing period.

◊ Analytical Results

	Measured Concentration (mg/l)†	
Day	Initial	24 hours
1	108	93.8
2	102	91.8
3	105	72.1
4	99.6	90.5
	Mean Concentrations* over all measurements 95.4 mg/l	
	Test material below limit of detection in controls	
	† Composite sample from all three vessels	

* Arithmetic mean

Results

- Nominal Concentrations: 0, 100 mg/l
- Measured Concentrations: 95.4 mg/l (mean over 96 hours of test)
- Units: mg./l.
- LC_{50}: >95.4 mg/l at 24, 48, 72 and 96 hours.
- LC_0: 95.4 mg/l at 24, 48, 72 and 96 hours
- NOEC: 95.4 mg/l at 24, 48, 72 and 96 hours

◊ Mortality: No fish died during the exposure period

◊ Sub-lethal Effects: No sub-lethal effects of the test substance were observed.

Conclusions

Remarks Field — The 96-hour LC_{50} for the limit test was >95.4 mg/l (based on measured concentrations). The no-observed-effect-concentration (NOEC) for the limit test was 95.4 mg/l.

Data Quality

- Reliability — Klimisch Code 1. May be used without restriction.

Reference — 1,3-Dioxolane CAS No. 646-06-0: Acute Toxicity to Bluegill, Lepomis macrochirus, Under Static-Renewal Test Conditions. Toxikon Corporation, Laboratory Project ID 00J0009g, October 2000.

Other

◊ This study is supported by an earlier study, sponsored by Celanese, in which Sheepshead minnows (Cyprinodon variegates, 5 per group) were exposed to Dioxolane at concentrations of 7500, 11000, 13000, 15000 and 25000 mg/l. In this study the 48 hour-LC_{50} was reported to be 12000 mg/l. and the 96-hour LC_0 was 7500 mg/l, the 96 hour-LC_{50} was reported to be 10000 mg/l. and the 96-hour LC_0 was 7500 mg/l. In this study 5 fish per concentration were exposed and a clear dose-response was established with a 24-hour mortality of 5/5 at 25000 mg/l. Based on the volatility of Dioxolane, the actual value for the EC_{50} in this study is likely somewhat than reported. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward bluegill sunfish 1

◊ The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour LC_{50} for fish to be 8150 mg/l.2

References for supporting studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Acute Toxicity to Aquatic Invertebrates

Type	Acute Toxicity to Aquatic Invertebrates
Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Purity 99.98%
Method	
• Guideline	OECD 202 (1984)
• Test Type	Daphnia, acute immobilization
• GLP	Yes
• Year	2000
• Analytical Procedures	Determination of test material concentration at 0, 24 and 48 hours
• Species/Strain	*Daphnia magna*
• Test Details	Static renewal
• Statistical Methods	None necessary due to lack of greater than 50% mortality

Remarks Field for Test Conditions	◊ Age at study initiation	Less than 24 hours
	◊ Test conditions	As specified in OECD 202.
	◊ Solvent	Moderately hard fresh water
	◊ Vessel	Crystallizing dishes 10 cm diameter by 5 cm deep. Vessels were kept covered with glass plate during test to reduce loss of test material. Dishes contained 200 ml of solution
	◊ Daphnids per group	Twenty
	◊ Daphnids per vessel	Five
	◊ Exposure period	48 hours
	◊ Temperature range	19.2 to 20.0º C
	◊ Observation times	24 and 48 hours
	◊ Solution pH range	7.6 to 7.8 at all concentrations at both 0 and 48 hours

◊ Dissolved oxygen Above 8.8 mg/l for all solutions at 0 and 48 hours

◊ Renewal Solutions were renewed after 24 hours

◊ Analytical Results

	Measured Concentration (mg/l)				
Target Conc	Initial	24 hours	24 hours Renewed	48 Hours	Mean Conc*
250	244	179	240	190	213
500	485	315	495	348	411
1000	982	546	990	570	772

* Arithmetic mean

◊ Water Parameters
- Hardness of 68 mg/l as calcium carbonate
- Alkalinity of 26 mg/l as calcium carbonate
- Specific conductivity of 527 microSiemens

Results

- Nominal Concentrations 0, 250, 500 and 1000 mg/l
- Measured Concentrations 213, 411 and 772 mg/l (mean over 48 hours of test)
  11
- Units mg./l.
- EC_{50} >764 at 24 hours
  > 772 at 48 hours
- EC_0
  - 24 hours: 764 mg/l
  - 48 hours: Not determined

Remarks Field for Results ◊ Immobilization

	Cumulative Number Immobilized	
Concentration	24 hours	48 hours
0	0	0
213	0	8/20
411	0	6/20
772	0	9/20

Conclusions

Remarks Field

- The EC_{50} (24 hour) was >764 mg/l and the EC_0 (24 hour) was 764 mg/l based on the mean measured concentrations. The EC_{50} (48 hour) was >772 mg/l and the and EC_0 was not found based on the mean measured concentrations
- The volatility of the test substance makes it difficult to accurately determine the exposure concentrations. Based on the lack of mortality and the lack of a typical dose-response curve, it appears that the stress of the renewal conditions may have contributed to the immobilization. It is clear that the test material has a low order of toxicity to daphnids. Because of this result and the difficulty in maintaining concentration levels, further studies were not conducted.
- A preliminary range-finding test was conducted as part of this study. In this test, ten daphnids were exposed for 48 hours to nominal concentrations of 0, 0.1, 1.0, 10.0, 100 or 1000 mg/l Dioxolane in water. Under these conditions, no immobilization was observed.

Data Quality

- Reliability — Klimisch Code 1. May be used without restriction.

Reference — 1,3-Dioxolane CAS No. 646-06-0: Acute Toxicity to the Water Flea, *Daphnia magna*, Under Static-Renewal Test Conditions. Toxikon Corporation, Laboratory Project ID 00J0009c October 2000.

Other

- This study is supported by an earlier study, sponsored by Celanese, in which Dioxolane was tested at 1000, 5000, 6500, 8000, 9000, 10000 and 12500 mg/l. In this study the 24-hour EC50 was reported to be 7650 mg/l and the 48-hour EC50 was reported to be 6950 mg/l.[1] In this study 20 daphnids were exposed and a clear dose-response relationship was obtained. Based on the volatility of Dioxolane, the actual value for the EC50 in this study is likely lower than reported. Nevertheless, this study supports the low order of toxicity found for Dioxolane toward daphnids.
- The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 48-hour LC_{50} for daphnia to be 7400 mg/l.[2]

References for supporting studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Toxicity to Aquatic Plants

Type	Toxicity to Aquatic Plants
None	1,3-Dioxolane CAS Number: 646-06-0 Purity 99.98%

Method

• Guideline	OECD 201
• Test Type	Algae Growth Inhibition
• GLP	Yes
• Year	2000
• Species/Strain	*Selenastrum capricornutum* The culture originated from an inoculum received from the Carolina Biological Supply Company (Burlington, NC) and has been maintained in the laboratory since December 3, 1999.
• Element Basis	Number of cells per ml. And area under the growth curve
• Exposure Period	72 hours
• Analytical Monitoring	Yes
• Statistical Methods	◊ EC_{50} values were calculated based on both biomass growth (comparison of area under the growth curves), the E_bC_{50}, and on the average specific growth rate, the E_rC_{50}. EC_{50} values and their 95 percent confidence limits were estimated by a computer program (U.S. EPA, 1994) for calculating EC values by probit analysis. ◊ In addition to the EC_{50} values, a no-observed-effect concentration (NOEC) was calculated by analysis of variance (ANOVA) with statistical differences between cell density means determined by Dunnett's procedure (U.S.EPA, 1988). Statistical differences were determined at a probability level of 0.05. ◊ Inhibition calculations are based upon a comparison of the areas under the growth curves and are reported using the symbol E_bC_{50}. The 24, 48 and 72-hour E_bC_{50} values and their 95 percent confidence limits were calculated.

Remarks Field for Test Conditions	◊ Test Temperature Range	The temperature ranged from 24.4 to 26.8º C.
	◊ Growth Medium Chemistry	The base water for the test medium was deionized water. The base water was enhanced with reagent-grade nutrients as described in ASTM (1994). The pH of the test medium was adjusted to 7.5 ± 0.1 prior to the addition of the test substance. [American Society for Testing and Materials (ASTM). 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. ASTM Designation E1218-90.]
	◊ Dilution Water Source	Deionized water from the Town of Jupiter Florida, supplemented as above.
	◊ Exposure Vessel	Sterile 250-mL glass Erlenmeyer flasks covered with gas exchange caps containing 100 ml of algal medium.
	◊ Stock Solutions Prepared	Approximately 1.0182 g of the chemical was brought to volume in a 100 ml volumetic flask with deionized water to prepare a stock concentration of 10,200 mg/l. The following amounts of stock (1.9, 3.75, 7.5, 15 and 30 ml) were used to make the test concentrations by mixing with 298.1, 296, 292.5, 285, and 270 ml of freshwater algal media individually.
	◊ Light Level and Quality	Lighting was continuous fluorescent lighting and intensity was measured daily at the surface of the test solutions during the 72-hour exposure period and ranged from 84 to 138 $\mu E/m^2/s$ as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor.
	◊ Test Design	▪ Replicates: three replicates for each test concentration. Six replicates were used for the dilution water control. ▪ Concentrations were determined by gc using a glp validated method. o Target: 0, 62.5, 125, 250, 500 and 1000 mg/l o Mean measured Control (<31.0) , 36.9, 81.0, 163, 280 and 877 mg/l.

◊ Analytical Determination of Test Material Concentrations

	Measured Concentrations mg/l			
Nominal Conc	Day-1	Day-3	Mean	Percent nominal
Control	ND	ND	---	---
62.5	60.6	13.1	36.9	59
125	124	37.9	81	64.8
250	262	64.7	163	65.2
500	520	39.3	280	56
1000	1027	726	877	87.7

◊ Method of calculating mean: Arithmetic based on composite samples of each replicate for each concentration at study initiation and study termination

◊ Exposure period: 72 hours

◊ Cell Counts: Algal growth was measured by direct cell count using a 0.1mm deep hemacytometer under a compound microscope. Algal counts were conducted on day one and approximately every 24 hours thereafter. Morphological observations were also conducted on the test treatment using a compound microscope to detect abnormal cell morphology and coloration as compared to the control replicates.

Results

- Nominal Concentrations: 0, 62.5, 125, 250, 500 and 1000 mg/l
- Measured Concentrations: (<31.0) , 36.9, 81.0, 163, 280 and 877 mg/l
- Units: mg./l.
- EC_{50}: The E_bC_{50} and E_rC_{50} (0-72 hours) were >877 mg /l.
- NOEC: 877 mg/l (72-hour)

Remarks Field for Results	◊	Biological Observations	After 72 hours of exposure to 1,3-Dioxolane, the percentage cell growth inhibition (based on area under the growth curve) compared to the control was 19% at the mean measured concentration of 877 mg/l. The growth curves of both the control and the test solution exhibited a pattern of exponential growth during the 72-hour growth period. Observations of cell morphology detected no changes in exposed cells as compared to cells in the control media. There was no significant statistical difference between the algal growth of the control and the test solutions
	◊	Daily Cell Counts From Each Replicate	These are presented to substantiate that there was no unusual variation between replicates associated with the possible selective volatilization of the test material from individual flasks.

Measured Conc (mg/l)	Cell Numbers (x 10^4)/ml			
	Replicate	24 hrs	48 hrs	72 hrs
Control	A	1.7	25	407
	B	2.1	36	378
	C	1.1	33	280
	D	0.9	43	358
	E	1.8	28	224
	F	1.9	26	289
36.9	A	1.3	13	318
	B	1.3	23	284
	C	2.2	16	218
81	A	1.9	33	329
	B	1.6	20	298
	C	0.9	48	333
163	A	0.4	30	304
	B	0.9	30	336
	C	1.8	56	189
280	A	1.7	29	324
	B	2.0	24	278
	C	1.3	40	291
877	A	2.1	33	229
	B	3.3	37	287
	C	2.1	34	211

◊ Mean Cell Density at Each Concentration at Each Time Point

Measured Conc (mg/l)	Mean Cell Numbers (x 10^4)/ml (s.d.)		
	24 hours	48 hours	72 hours
Control	1.6 (0.475)	32 (6.91)	323 (69.4)
36.9	1.6 (0.520)	17 (5.13)	273 (50.8)
81	1.5 (0.513)	34 (14.0)	320 (19.2)
163	1.0 (0.709)	39 (15.0)	276 (77.3)
280	1.7 (0.351)	31 (8.18)	298 (23.7)
877	2.5 (0.693)	35 (2.08)	242 (39.7)

◊ Percent Inhibition

Measured Conc (mg/l)	Percent Inhibition		
	0-24hrs	24-48 hrs	48-72 hrs
36.9	0	-47	-21
81	-17	6	0
163	-100	18	-9
280	17	-2	-7
877	150	15	-19

Conclusions

Remarks Field

The E_bC_{50} and E_rC_{50} (0-72 hours) were >877 mg /l (based on measured concentrations). The 72-hour no-observable-effect concentration (NOEC) was 877 mg/l.

The test material was somewhat volatile; however, sufficient Dioxolane remained in the culture flasks (especially at the highest concentration tested) to provide a valid estimate of the growth inhibition potential of the test material to green algae.

Data Quality

- Reliability

Klimisch Code 1. Reliable without restriction. Study was conducted in accord with current OECD guideline under glp conditions. Analytical measurements verified exposure concentrations.

References

1,3-Dioxolane: Toxicity to The Freshwater Green Alga, *Selenastrum capricornutum*, Under Static Test Conditions. Toxikon Laboratories, Jupiter FL, Project ID 00J0009b, 27 September 2000, submitted to and sponsored by Ticona Corporation and Ferro Corporation.

Other

This study is supported by an earlier study, sponsored by Celanese, in which Trioxane was tested for growth inhibition of *Selenastrum capricornutum*. In this study, algae growth was measured out to 14 days of exposure at levels of 1000, 5000 or 10000 mg/l with counts recorded on days 3, 6, 10 and 14. Significant inhibition was seen only at 5000 mg/l and above 1000 mg/l was determined to be the NOEC. Graphically, the 96-hour EC_{50} can be determined to be in the range of 4000 mg/l; however, loss of test material may affect this estimate[1]

The EPA ECOSAR Modeling Program found in EPIWIN, estimates the 96-hour EC_{50} for green algae to be 4075 mg/l.[2]

References for supporting studies

1. Report to Celanese Chemical Company Inc. on Toxicology and Fate of Selected Industrial Chemicals in Aquatic Ecosystems. J.R. Walton and E.M. Davis, University of Texas at Houston. December 1980.
2. ECOSAR modeling program, version 0.99f, as found in EPIWIN v 3.05, Syracuse Research Corporation, Syracuse NY (April 2000).

Acute Oral Toxicity

Type	Acute Oral Toxicity
Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Commercial Grade

Method

• Guideline	Based on methods described in 16 CFR 1500.3 (c) Consumer Products Safety Commission: Federal Hazardous Substance Act
• GLP	No
• Year	1980
• Species	Rat
• Strain	Albino Rats (Sprague-Dawley CD®)
• Route of administration	Oral Gavage
• Doses	2500, 3500, 5000, 7100, 10000 mg/kg
• Sex	Males and Females
• Number of Animals/group	Five
• Vehicle	None, administered neat

Remarks Field for Test Conditions	◊ Age at Study Initiation	Unknown, Young Adults At study initiation males weighed 265 to 289 grams and females weighed 233 to 245 grams
	◊ Volume administered	1.3 to 9.2 ml/kg body weigh
	◊ Post-dose observation period	14 Days

Results

- LD_{50} — LD-50 = 5.2 g/kg with 95% confidence limits of 4.3 to 6.1 g/kg
- Number of deaths at each dose level

Dose Level mg/kg	Mortality, males	Mortality, females
2,500	0/5	0/5
3,500	2/5	0/5
5,000	1/5	2/5
7,100	5/5	5/5
10,000	5/5	5/5

Remarks Field for Results

◊ Time of death

Dose mg/kg	Males (d= day)	Females
3500	d-1, d-1	none
5000	4 hours	d-1, d-1
7100	2 hours, d-1, d-1, d-2, d-2	1 hour, 4 hours, d-1, d-1, d-1
10,000	2 hours, 4 hours, 6 hours, 6 hours, d-1	1 hour, 2 hours, 2-hours, 4 hours, 4-hours

◊ Clinical Signs

Dose mg/kg	Clinical Signs
2,500	• Ataxia, 1 female from 2 to 4 hours after dosing. • Respiratory rate decrease, 3 animals, 2 to 4 hours • Motor activity decrease, all animals 1 to 4 hours.
3,500	• Ataxia, 2 females 2 or 4 hours after dosing. • Fine tremors, 1 male, 4-hours after dosing only • Respiratory rate decrease, 5 animals, 2 to 4 hours • Motor activity decrease, 9/10 animals starting at 1 hour, decreasing to 2 animals at 24 hours. • Piloerection, 3 animals, 2 hours to day-2 • Prostration, 3 animals, from 2 to 4 hours.
5,000	• Ataxia, 1 male, only at 2 4 hours after dosing. • Fine tremors, 1 female 1- 4 hours after dosing • Respiratory rate decrease, 5 animals, 2 to 4 hours • Motor activity decrease, 9 animals starting at 1 hour, decreasing to 4 animals at 24 hours. • Prostration, 5 animals total, various times from 1 to 24 hours. • Hypothermia, 2 animals at 4 or 24 hours after dosing
7,100	• Respiratory rate decrease, 8 animals total, various 1 to 24 hours after dosing • Motor activity decrease, 1 animal 1-2 hours after dosing. • Prostration, 8 animals total, various times from 1 to 24 hours. • Hypothermia, 4 animals at 4 or 24 hours after dosing
10,000	• Respiratory rate decrease, 6 animals total, various 1 to 4 hours after dosing • Labored breathing, 2 animals at 1 hour after dosing only • Prostration, 9 animals total, various times from 1 to 4 hours. • Hypothermia, 1 animal at 2 hours after dosing

	◊ Necropsy Findings	Necropsy only conducted on animals dying less than 24 hours after dosing and limited to determining if gavage was the cause of death.
	◊ Target Organs	No potential target organs were identified in report, CNS may have been affected by solvent narcosis.
	◊ Sex Differences	Effects on males and females were similar.

Conclusions

Remarks Field	The oral LD_{50} in the rat was determined to be 5,200 mg/kg. Solvent induced narcosis may have been the cause of deaths occurring on day-1. Study documentation is good. Results are consistent with other data for the material, including literature studies.

Data Quality

• Reliability	Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References	Acute Oral Toxicity Study in Rats, Bio/dynamics Inc., Project No. 6004-79 (06/05/80), Sponsored by The Celanese Corporation, New York, NY.
Other	This study is supported by a study in the literature that reports the acute oral LD_{50} in the rat to be 5,800 mg/kg[1]. This study is also supported by a limit test, conducted by Hoechst, that reported the LD_{50} to be greater than 2000 mg/kg[2]

References for supporting studies

1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Med Pr; Vol 38, 1987, P184-90.
2. 1,3-Dioxolan - Profung der akuten oralen Toxizitatan der Wistar Ratte. Hoechst AG Pharma Research Toxicology Report No. 87.1441, September 1987.

Acute Inhalation Toxicity

Type	Acute Inhalation Toxicity	
Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Commercial Grade	
Method		
• Guideline	None specified	
• GLP	No	
• Year	1980	
• Species	Rat	
• Strain	Charles River CD®	
• Route of administration	Whole-body inhalation as vapor	
• Doses	0, 37.9, 60.6, 67.9, 88.4 and 201.9 mg/l (nominal concentrations)	
• Sex	Males and Females	
• Exposure Period	Four hours	
• Number of Animals/group	Five animals of each sex per dose level	
• Vehicle	Air	
Remarks Field for Test Conditions	◊ Age at Study Initiation	Unknown, Young Adults At study initiation males weighed 204 to 295 grams and females weighed 211 to 273 grams
	◊ Doses	Initial dose was 201.9 mg/l (nominal) which caused 100% mortality. Second group was 37.9 mg/l, which caused 0% mortality. Additional groups were added until enough data were accumulated to calculate an accurate $LC_{50.}$
	◊ Post-dose observation period	14 Days
Results		
• LC_{50}	68.4 mg/l (95% confidence interval of 61.0 – 766. mg/l)	

- Number of deaths at each dose level

Concentration (mg/l)	Number of deaths		
	Males	Females	Total
0	0	0	0
37.9	0	0	0
60.6	0	1	1
67.9	4	1	5
88.4	4	5	9
201.9	5	5	10

Remarks Field for Results

◊ Time of death

Conc. (mg/l)	Males and Females (d=day, h=hour)
60.6	6h
67.9	5h, 8h, d1, d1, d1
88.4	4h, 4h, 5h, 5h, 6h, 7h, d1, d1, d1
201.9	All ten within 2 hours

Clinical Signs

37.9 mg/l

Sign	Onset	Duration	# Animals
Lacrimation	30 min	1 day	Most
Labored breathing	2 hours	3 hours	Most
Prostrate	3 hours	3 hours	All
No response to auditory stimuli	3 hours	1 hour	Most
Loss of muscle tone coordination	45 min	< 24 hours	Most

Clinical Signs

60.6

Sign	Onset	Duration	# Animals
Lacrimation	30 min	2 days	Most
Labored breathing	2 hours	6 hours	Some
Rapid breathing	3 hours	3 hours	All
Reduced activity	30 min	< 24 hours	All
Prostrate	2 hours	3 hours	All
No response to auditory stimuli	2 hours	3 hours	All
Loss of muscle tone coordination	1 hour	2 hours	Most

Clinical Signs

67.9 mg/l

Sign	Onset	Duration	# Animals
Lacrimation	15 min	2 days	Most
Labored breathing	2 hours	6 hours	Half
Rapid breathing	3 hours	<24 hours	All
Reduced activity	15 min	< 24 hours	All
Prostrate	2 hours	3 hours	All
No response to auditory stimuli	45 min	3 hours	All
Loss of muscle tone coordination	45 min	30 min	Most

Clinical Signs

88.4 mg/l

Sign	Onset	Duration	# Animals
Lacrimation	15 min	< 24 hours	Most
Labored breathing	4 hours	< 24 hours	Many
Rapid breathing	2 hours	3 hours	All
Reduced activity	15 min	< 24 hours	All
Prostrate	2 hours	4 hours	All
No response to auditory stimuli	60 min	4 hours	All
Loss of muscle tone coordination	45 min	2 hours	Most

Clinical Signs
201.9 mg/l

Sign	Onset	Duration	# Animals
Lacrimation	15 min	< 2 hours*	All
Labored breathing	45 min	< 2 hours*	All
Salivation	14 min	< 2 hours*	All
Reduced activity	15 min	< 2 hours*	All
No response to auditory stimuli	45 min	< 2 hours*	All
Dead	< 2 hours		All

* Animals died after 2 hours of exposure

Body Weights Group	Day Mean Body Weights (grams)						
		0	1	2	4	7	14
Control	Males	282	285	285	297	314	345
	Females	227	225	228	231	236	244
37.9 mg/l	Males	285	264	263	276	290	318
	Females	255	240	244	244	255	262
60.6 mg/l	Males	259	232	242	254	268	303
	Females	220	203	209	214	222	226
67.9 mg/l	Males	248	231	224	242	264	311
	Females	223	200	208	216	228	241

Necropsy Findings

Group	Finding	Number
Controls	Mottled lungs	8/10
37.9 mg/l	Mottled lungs	2/10
60.6 mg/l	Lung, red foci	2/10
	Mottled lungs	1/10
67.9	Mottled lungs	6/10
	Liver purple	2/10
88.4	Mottled lungs	10/10
	Liver discolored	10/10
	Spleen dark red	1/10
201.9 mg/l	Mottled lungs	10/10
	Lungs blood filled	3/10
	Liver discolored	6/10

Other — Necropsy findings also included gastrointestinal gas distention in animals dying prior to sacrifice. Bladders distended with fluid were also observed in these early deaths.

Conclusions

Remarks Field

An LC_{50} of 68.4 mg/l was determined using a series of concentrations. Solvent induced narcosis appeared as a common effect and may have been the cause of death at the high concentration. Lung and liver abnormalities were common and severity was dose related.

Data Quality

- Reliability

Klimisch Code 2 Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References

An acute inhalation toxicity study of C-121 [1,3-Dioxolane] in the rat. Project No. 79-7304, Bio/dynamics Inc., July 11, 1980.

Other

This study is supported by a literature study that reported an LC_{50} of 118 mg/l in the rabbit (exposure time not given), 87 mg/l/ (4 hours) in the male rat and 166 mg/l (4 hours) in the guinea pig.[1]

References for supporting data

1. Czajkowska, T, Krysiak, B and Popiânska, E. Experimental studies of toxic effects of 1,3,5-trioxane and 1,3-dioxolane. I. Acute toxic effect. Med Pr; Vol 38, 1987, P184-90.

Repeated Dose Toxicity, Two-week Gavage

Type Repeated Dose Toxicity, Two-week Inhalation

Test Substance

1,3-Dioxolane

CAS Number: 646-06-0

Purity listed as 99.9%

Method

- Guideline
  - ◊ Toxic Substances Control Act Test Guidelines - 798.4900
  - ◊ Meets OECD 412 Guideline except for lack of clinical chemistry determinations
- GLP: Yes
- Year: 1991
- Species: Rat
- Strain: Crl:CD®(SD)BR, Charles River
- Route of administration: Gavage in corn oil.
- Duration of Test: Fifteen days
- Doses: Water control, vehicle control, 75, 250, 750 and 2000 mg/kg/day
- Sex: Rats of each sex.
- Frequency of Treatment: Daily for fourteen consecutive days.
- Number of Animals/group: Ten of each sex
-
- Control Group and Treatment: Water treated controls and corn oil treated controls. (Ten animals of each sex per group)
- Post-Exposure Observation Period: Overnight

- Statistical Methods
  - ◊ All hematology data were statistically compared to both control groups using a one-way Analysis of Variance Test and then Dunnett's Test, to determine the statistical significance of the individual groups in comparison with each of the control groups
  - ◊ All body weight, feed consumption and organ weight data were statistically compared against both control groups using the following statistical tests: Analysis of Variance, Dunnett's Test, Bartlett's Test of homogeneity, Kruskal-Wallis Test and Dunn's Test.

Remarks Field for Test Conditions

◊	Age at study initiation	Males: approximately nine weeks Females: approximately nine weeks
◊	Number animals of each sex per dose	Ten of each sex per concentration
◊	Satellite groups	None
◊	Housing	Individual wire-bottom cages
◊	Clinical observations performed and frequency	Mortality twice daily and gross signs daily about 30 minutes after dosing. Body weights daily. Feed consumption weekly.
◊	Terminal observations	▪ Blood samples were evaluated for the following parameters: red blood cell count, hematocrit, hemoglobin, mean corpuscular volume, white blood cell count, white blood cell differential count, red blood indices for mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, reticulocyte counts (absolute), red blood cell morphological assessment and platelet counts (absolute). ▪ Postmortem examination included gross lesions of the thoracic, abdominal and pelvic viscera. organ weights were recorded for the liver, kidneys, adrenals, spleen, pancreas, lungs, pituitary, thymus, as well as the reproductive organs (male rats - testis, epididymides and accessory sex glands; female rats - ovaries and uterus) .

- ◊ Histopathology
  - ▪ Histopathology was performed on all tissues showing gross changes, liver, kidneys, adrenals, spleen, pancreas, lungs, pituitary, thymus, as well as the reproductive organs (male rats - testis, epididymides and accessory sex glands; female rats - ovaries and uterus). Initially, tissues form both controls and the high-dose were examined. Because this preliminary examination of tissues from both control groups and the 2000 mg/kg/day dosage group revealed potential histopathology in the kidney, liver, thymus (male and female rats) and testes (male rats), these tissues were also examined in the remaining dosage groups.
- ◊ Differences from OECD Guideline
  - ▪ This study is in accord with the current OECD 412 guideline for 14 or 28-day inhalation studies with the exception that clinical chemistry parameters were not determined. The current study uses twice the number of animals required by the OECD guideline and one additional dose level.

Results

- NOAEL
  - ◊ Males: 75 mg/kg/day
  - ◊ Females: 75 mg/kg/day
- LOEAL
  - ◊ Males: 250 mg/kg/day
  - ◊ Females: 250 mg/kg/day
- Mortality
  - ◊ Males: Three high dosage group male rats were found dead on days 4, 9 and 14 of the study. Deaths were preceded by clinical observations, and two of these rats had severe weight losses. Necropsy of one rat revealed gastric erosions. No other deaths occurred during the conduct of the study.
  - ◊ Females: Four 2000 mg/kg/day dosage group female rats died. These deaths occurred on days 3, 8, 9 and 10 of the study and were preceded by clinical observations. These rats had moderate, although sometimes transient, weight losses occur, and necropsy of one rat revealed reddened areas in the stomach. Two additional high dosage group deaths occurred on days 10 and 13 of the study; these deaths were related to intubation accidents. No other deaths occurred during the conduct of the study.

- Toxic Responses

Dose (mg/kg/day)	Toxic Response (statistical comparisons noted are against the corn oil control)
75	▪ Males: No significant effects ▪ Females: No significant effects
250	▪ Males ○ Slight reduction in platelets ($p>.05$) ○ Reduction in body weight gain ○ Reduced feed consumption ▪ Females ○ Reduction in lymphocyte count($p\leq.01$)
750	▪ Males ○ Reduction in platelets ($p\leq.01$) ○ Reduction in body weight gain ○ Reduced feed consumption ○ Liver and lung relative weights increased ○ Reduced relative thymus weight ▪ Females ○ Excess salivation in some females. ○ Reduction in lymphocyte ($p\leq.05$) and reticulocyte count ($p\leq.01$) ○ Liver relative weight increased ○ Spleen and thymus relative weights decreased

2000	▪ Males ○ Clinical observations after dosing included hypotonia, excess salivation, ataxia, decreased motor activity, rales, impaired and lost righting reflex, labored breathing, red penile exudate and urine-stained abdominal fur ○ Reduced feed consumption ○ Reduction in platelets ($p \leq .01$) ○ Overall loss in body weight. ○ Liver, lung and kidney relative weights increased ○ Prostate, spleen and thymus weights decreased ○ Histopathological findings in liver (6/10), kidney (4/10), thymus (6/10) and testis(2/10). ▪ Females ○ Clinical observations after dosing included excess salivation (750 and 2000 mg/kg/day), hypotonia, ataxia, decreased motor activity, impaired and lost righting reflex, gasping, bradypnea, oral exudate, rales, lacrimation and urine-stained abdominal fur ○ Reduction in lymphocyte count ($p \leq .01$) ○ Reduction in platelet count ($p \leq .01$) ○ Reduction in body weight gain ○ Liver weight marginally increased ○ Kidney relative weight increased ○ Spleen weight marginally decreased ○ Thymus weight decreased ○ Pancreas relative weight deceased ○ Histopathological findings in liver (5/10), kidney (3/10), thymus (5/9) and spleen (2/10)

Remarks Field for Results ◊ Body Weights Gains

	Mean Body Weight Gains (grams)			
Dose	Males		Females	
(mg/kg)	Day 1-8	Day 8-15	Day 1-8	Day 8-15
Water only	29.5	33.8	11.0	12.6
Corn Oil	28.5	38.5	8.6	11.4
75	27.5	37.4	11.5	9.5
250	26.7	32.6	5.2	10.8
750	20.5	31.0	4.2	16.0
2000	-29.0	9.3	-10.1	11.0

- ◊ Clinical Signs
  - ○ Males: Clinical observations after dosing included hypotonia, excess salivation, ataxia, decreased motor activity, rales, impaired and lost righting reflex, labored breathing, red penile exudate and urine-stained abdominal fur.
  - ○ Females: Clinical observations attributable to the 750 and 2000 mg/kg/day dosages of the test substance included excess salivation (750 and 2000 mg/kg/day), hypotonia, ataxia, decreased motor activity, impaired and lost righting reflex, gasping, bradypnea, oral exudate, rales, lacrimation and urine-stained abdominal fur (2000 mg/kg/day).
- ◊ Hema-tology
  - ○ Group designations are used to describe the hematology results, groups are: Group I, water control; Group II, corn oil control; Group III, 75 mg/kg/day; Group IV, 250 mg/kg/day; Group V, 750 mg/kg/day; Group VI, 2000 mg/kg/day.
  - ○ Among male animals, statistically significant differences were seen only in the platelet counts. Groups IV, V, and VI differed significantly from group I at the 0.01 level. Groups V and VI differed significantly from group II at the 0.01 level, but the difference between groups II and IV was not statistically significant

◊ Hematology

○ Among female animals, statistically significant differences were seen in the total leukocyte count, lymphocyte count, platelet count, nucleated red blood cells, and reticulocyte count. In the total leukocyte count, groups IV, V, and VI differed significantly from group I at the 0.05 level; none of the treated groups differed significantly from group II. With respect to lymphocytes, groups IV, V, and VI differed significantly from group I at 0.01; group IV and VI differed significantly from group II at 0.01 while group V differed significantly from group II at 0.05. No significant differences occurred among groups with respect to neutrophils or minor cell types indicating that the differences in total leukocyte count was a function of differences in lymphocytes. No corresponding effect was evident in males. Nucleated red blood cells were significantly different in groups III, V, and VI from group I at 0.05 but did not differ significantly from group II. Reticulocytes were significantly higher in group VI than in group I at 0.05, and lower in group IV than in group II at 0.01. No other differences among groups were significant with respect to reticulocytes. It appears that the differences in nucleated red blood cells and reticulocytes, cells of the same lineage differing only with respect to maturity, are a function of random variation rather than a treatment effect, particularly because differences from controls occur in both directions. In female animals platelets in group VI were significantly lower than in group I and group II at the 0.01 level.

○ From the above data, it appears that a decrease in circulating platelets was a treatment effect as it occurred at a statistically significant levels in both males and females. In males, rats in groups IV, V, and VI had significantly lower platelet counts than group I and groups V and VI had significantly lower platelet counts than group II. In females group VI animals had significantly lower platelet counts than groups I and II. Among the other groups there was a non-significant dose-related trend.

◊ Hematology Comments

Treatment related hematologic changes observed in this study were a decrease in platelets, seen in both sexes, and a decrease in lymphocytes (also reflected in total leukocytes) seen in females only. No hematologic treatment effects were observed in the rats given 75 mg/kg/day of the test compound.

◊ Organ Weights	Treatment related organ weight changes are given above under toxic effects. Organ weigh changes were not remarkable although some reached statistical significance.
◊ Necropsy findings	Examination of each animal during necropsy did not indicate any exposure-related gross pathologic changes
◊ Histopath-ology	No treatment-related microscopic changes were observed in any of the male and female rats given 75, 250, or 750 mg/kg/day of 1,3-Dioxolane. Treatment-related microscopic changes were observed in the liver, kidney, thymus, and testis of rats given 2000 mg/kg/day of 1,3Dioxolane. The treatment -related changes consisted of centrilobular. hepatocellular hypertrophy and midzonal hepatocellular vacuolation (male and female rats); thymic atrophy (male and female rats); renal cortical tubular basophilia and dilatation and accumulations of birefringent intratubular crystals (male rats); subacute renal pyelitis (male and female rats), and multifocal testicular degeneration. Incidence values are given above.

Conclusions

Remarks Field

Gavage administration of Dioxolane to rats of each sex was associated with hematologic effects, body weight gain reductions and organ weight changes at doses of 750 and 2000 mg/kg/day. The 250 mg/kg/day dose level was associated with decreases in platelets in male rats and lymphocyte counts in female rats.

The authors of the laboratory report elected to call 250 mg/kg/day a NOEL in the female rat. The writer of this summary is not in agreement with that conclusion and has selected 75 mg/kg/day as the appropriate NOEL.

Data Quality

- Reliability

Klimisch Code 1. May be used without restriction.

References

Repeated Dose Oral Toxicity Study of 1,3-Dioxolane Administered Via Gavage to Crl:CD®(SD)BR Rats. Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044, Laboratory Project ID 508-002P.

Other

◊ This study is supported by a two-week inhalation study of Dioxolane conducted by BioDynamics for Celanese Corporation. In this study Charles River CD rats (5/sex/group) were exposed for six hours per day, five days per week, for two weeks at concentrations of 0, 984 or 3280 ppm. All animals survived the duration of the study. No treatment related observations were recorded. Body weights, organ weights, clinical chemistry parameters, and macropathology were unremarkable in treated animals. Depressed leukocyte values in male and female exposed animals were reported.[1]

◊ This study is supported by a 12-day inhalation study of Dioxolane conducted by Dow Chemical. The primary effect of Dioxolane inhalation exposure to rats was a reduction in white blood cells counts at 2319 or 5132 ppm. Body weight gain was affected at the 5132-ppm exposure level. There was no corresponding myeloid toxicity or inflammatory foci[2].

References for supporting studies

1. A two week inhalation toxicity study of C-121 (1,3-Dioxolane) in the rat. Bio/Dynamics Inc Project No. 80-7429, June 22, 1981.
2. 1,3-Dioxolane: A two-Week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company 1989 Study ID K-010634-005

Repeated Dose Toxicity, Four-week Drinking Water

Type	Repeated Dose Toxicity, Four-week Oral
Test Substance	1,3-Dioxolane CAS Number: 646-06-0
Method	
• Guideline	None
• GLP	No
• Year	1977
• Species	Rat, mouse, golden Syrian hamster.
• Strain	Charles River strain
• Route of administration	Drinking water
• Duration of Test	Four weeks
• Doses	0, 0.5, 1.0 and 2.0% in drinking water
• Sex	Male and Female
• Exposure Period	Twenty-four hours per day
• Frequency of Treatment	Continuous, seven days a week.
• Number of Animals/group	Five of each sex
• Control Group and Treatment	Five animals of each sex exposed only to water
• Post-Exposure Observation Period	None
• Statistical Methods	Body weight data analyzed by ANOVA followed by Tukey's or Scheffe's test of multiple comparison.

Remarks Field for Test Conditions	◊ Age at study initiation	Not specified
	◊ Number animals of each sex per dose	Five of each sex per concentration
	◊ Satellite groups	None

- ◊ Housing — Individual
- ◊ Clinical observations performed and frequency
  - Mortality daily.
  - Body weights weekly.
  - Water consumption twice weekly.
- ◊ Terminal observations — Body weights
- ◊ Test Substance — Prepared weekly in tap water.

Results

- NOAEL
  - ◊ Rats
    - Males: 0.5%
    - Females: 0.5%
  - ◊ Mice
    - Males: 0.5%
    - Females: 0.5%
  - ◊ Hamsters
    - Males: 1.0 %
    - Females: 1.0%
- LOEAL
  - ◊ Rats
    - Males: 1.0%
    - Females 1.0%
  - ◊ Mice
    - Males: 1.0%
    - Females 1.0%
  - ◊ Hamsters
    - Males: 2.0%
    - Females 2.0%
- Mortality — All animals survived the duration of the study.
- Toxic Responses

Dose	Toxic Responses		
0.5%	Reduced water consumption for female rats only.		
1.0%	▪ Rats	Males	Reduced water consumption Reduction in body weight gain†*
		Females	Reduced water consumption Slight reduction in body weight gain

	▪ Mice.	Males	Slight reduction in body weight gain
		Females	Reduced water consumption Slight reduction in body weight gain
	▪ Hamsters	Males	None
		Females	None
2.0%			
	▪ Rats	Males	Reduced water consumption Reduction in body weight gain††
		Females	Reduced water consumption Reduction in body weight gain†
	▪ Mice	Males	Slight reduction in body weight gain
		Females	Reduced water consumption Slight reduction in body weight gain
	▪ Hamsters	Males	Slight reduction in body weight gain
		Females	Slight reduction in body weight gain
	† = p≤ 0.05 †† = p≤ 0.01 * Although absolute body weights were not significantly reduced, body weight gain was.		

Remarks Field for Results

◊ Body Weights

Mean Body Weights (n=5)

Drinking Water Level (percent)	Rats Body Weight on Week (grams)				
Males	0	1	2	3	4
0	210	271	331	376	391
0.5	209	264	314	351	364
1.0	210	256	298	332	334
2.0	209	237	273†	304†	309††
Females					
0	156	182	209	231	238
0.5	157	178	203	221	227
1.0	156	182	196	208	215
2.0	157	164	182	198†	193†

† = p≤ 0.05 †† = p≤ 0.01

◊ Body Weights

Drinking Water Level (percent)	Mice Body Weight on Week (grams)				
Males	0	1	2	3	4
0	27	33	34	37	38
0.5	28	32	34	36	37
1.0	28	32	35	36	37
2.0	28	30	32	33	34
Females					
0	23	27	28	29	30
0.5	23	26	28	28	28
1.0	23	26	27	27	29
2.0	23	24	25	26	27

◊ Body Weights

Drinking Water Level (percent)	Hamsters Body Weight on Week (grams)				
Males	0	1	2	3	4
0	69	80	93	99	104
0.5	69	84	96	104	107
1.0	70	77	90	99	105
2.0	69	77	85	92	95
Females					
0	64	71	80	88	93
0.5	63	74	86	94	100
1.0	64	74	83	90	98
2.0	64	72	79	83	86

◊ Clinical Signs

None recorded

Conclusions

Remarks Field

- Rats of each sex showed reduced body weigh gains at the two highest dose levels. Body weight gains of high-dose mice and hamsters also appear to be affected but did not achieve statistical significance. The authors of the report only noted the statistically significant changes in body weight gains for rats (males 1.0 and 2.0 %, females 2.0%). Hamsters appear to be less sensitive to the toxic effects of dioxolane than rats and mice and rats appear to be the most sensitive based solely on body weigh gains.
- The dosing for the male rats at 1% drinking water with water consumption at 30 ml per day calculates to approximately 1000 mg/kg/day Dioxolane. This is the approximate dose level were body weight changes are observed in other studies.

Data Quality

- Reliability: Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References: Four-week pilot study with Dioxolane in drinking water of albino rats, albino mice and golden Syrian hamsters. Industrial BIO-Test Laboratories, Inc., Project No. 8560-10579, July 12, 1977.

Other: This study is supported by a 14-day corn-oil gavage study in rats of each sexing which hematologic effects, body weight gain reductions and organ weight changes were reported at doses of 750 and 2000 mg/kg/day. The 250-mg/kg/day dose did not affect body weigh gain.

References for supporting studies: Repeated Dose Oral Toxicity Study of 1,3-Dioxolane Administered Via Gavage to Crl:CD®(SD)BR Rats. Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044, Laboratory Project ID 508-002P.

Repeated Dose Toxicity, Two-week Inhalation

Type	Repeated Dose Toxicity, Two-week Inhalation
Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Purity 99.99 % by analysis

Method

• Guideline	This study was conducted to meet the requirements of the United States Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards in 40 CFR part 792; TSCA Health Effects Test Guideline in 40 CFR 798.250; and OECD ISBN 92-64-12367-9 Paris 1982. Study also meets OECD 412 Guideline.
• GLP	Yes
• Year	1989
• Species	Rat
• Strain	Fischer 344 Charles River, Kingston NY
• Route of administration	Whole body inhalation of vapor
• Duration of Test	Twelve days
• Doses	0, 516, 2319 or 5132 (Mean, measured concentrations)
• Sex	Male and Female
• Exposure Period	Six hours per day
• Frequency of Treatment	◊ Five days a week ◊ Nine treatments
• Number of Animals/group	Five of each sex
•	
• Control Group and Treatment	Five animals of each sex exposed only to air under the same chamber conditions
• Post-Exposure Observation Period	Overnight

- Statistical Methods
  - ◊ Descriptive statistics (mean and standard deviation), were used to report chamber concentrations, temperature, and relative humidity.
  - ◊ Remaining parameters were first tested for equality of variance using Bartlett's test. If the results for Bartlett's test rejected the equality of variances, the parameter was flagged for careful evaluation of results. All parameters were then subjected to appropriate parametric analysis as described below. In-life body weights, hematologic (excluding differential WBC) and clinical chemistry parameters, terminal body weights, and organ weights (absolute and relative, except testis) were evaluated using a two-way analysis of variance (ANOVA) with the factors of sex and dose. Results for absolute and relative testis weights were analyzed using a one-way ANOVA. If significant dose effects were determined in the one-way ANOVA, then separate doses were compared to controls using Dunnett's test. For those parameters examined by a two-way ANOVA, examination was made first for a significant sex-dose interaction. If this existed, a one-way ANOVA was done separately for each sex. If no sex-dose interaction was identified, and a dose effect was identified, or if in the subsequent ANOVAs separated by sex a dose effect was identified, the separate ANOVAs were used for each exposure group with control. To control for multiple comparisons with control, a Bonferroni correction was used.

Remarks Field for Test Conditions

- ◊ Age at study initiation
  - Males: eight weeks
  - Females: eight weeks
- ◊ Number animals of each sex per dose: Five of each sex per concentration
- ◊ Satellite groups: None
- ◊ Housing: Not specified in report
- ◊ Clinical observations performed and frequency
  - Mortality and gross signs daily prior to exposure.
  - Body weights on days 1, 3, 5, 8, and 11.
  - Behavior pattern and nervous system activity were assessed by specific observations for lethargy, tremors, convulsions, salivation, lacrimation, diarrhea, and other sign of altered nervous system function.

◊ Terminal observations	▪ Blood taken for hematology (red cell count, hemoglobin, hematocrit, total white blood cell count, platelets, differential leukocyte counts and morphology of WBCs, RBCs and platelets) and clinical chemistry (urea nitrogen, alanine aminotransferase activity, aspartate aminotransferase activity, alkaline phosphatase activity, glucose, total protein, albumin, globulin, total bilirubin, cholesterol, triglycerides, phosphorus and calcium) ▪ Complete gross postmortem examination. ▪ Organ weights for brain, heart, lungs, liver, kidneys, and testis were recorded for all animals.
◊ Histopathology	▪ The following tissues were examined from all control and high-exposure animals only. Adrenals, aorta, bone, bone marrow, brain (cerebrum, brainstem, cerebellum), cecum, cervix, coagulating glands, colon, duodenum, epididymides, esophagus, eyes, gross lesions, heart, ileum, jejunum, kidneys, lacrimal/harderian glands, larynx, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissues, mesenteric lymph node, mesenteric tissues, nasal tissues, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid-gland, tongue, trachea, urinary bladder, uterus, vagina ▪ Microscopic examination of tissues from rats in lower 1,3-dioxolane exposure groups was not conducted since no target organs were identified in the high-exposure group.
◊ Differences from OECD Guideline	This study is in accord with the current OECD 412 guideline for 14 or 28-day inhalation studies. There are slight differences in the suggested parameters for clinical chemistry and in the duration of the study (12-days) but these are not considered scientifically significant.

Results

- NOAEL
  - ◊ Males: 516 ppm
  - ◊ Females: 516 ppm

- LOEAL
  - ◊ Males: 2319 ppm
  - ◊ Females: 2319 ppm

- Mortality

  All animals survived the duration of the study.

- Toxic Responses

Dose	Response
516 ppm	▪ No significant effects
2319 ppm	▪ Reduction in white-blood cell counts for males and females. ▪ Decrease in spleen weight in females ▪ Increased relative liver weight in females
5132 ppm	▪ Reduction in white-blood cell counts for males and females. ▪ Deceased body weight gains for males (11%) and for females (4%) ▪ Decreased alertness and slight incoordination at the end of each exposure lasting 45 to 85 minutes. ▪ Other changes detailed below were not considered toxicologically significant.

Remarks Field for Results

◊ Body Weights

	Body Weight on Day (grams)				
Males	1	3	5	8	11
Control	136	146	154	166	184
516 ppm	135	146	150	168	176
2319 ppm	134	142	150	165	175
5132 ppm	135	134	139	156	164
Females					
Control	112	117	118	124	129
516 ppm	113	118	120	129	132
2319 ppm	114	118	119	128	131
5132 ppm	112	110	111	121	124

◊ Clinical Signs

Animals exposed to 5132 ppm dioxolane appeared to have decreased alertness and incoordination at the end of each exposure lasting 45 to 85 minutes.

◊ Hematology WBC

	Mean White Blood Cells ($x10^3$)	
Conc (ppm)	Males	Females
Control	9.5±0.9	7.0±2.1
516 ppm	9.1±0.8	7.0±1.7
2319 ppm	6.9±0.9	5.2±0.7
5132 ppm	5.8±1.4	4.6±1.2

◊ Hematology Comments	▪ The mean RBC, HGB, HCT and PLAT values of males and females exposed to 2319 and 5132 ppm were statistically increased when compared to mean control values. These slight increases were exposure-related but were not considered toxicologically significant. Historical control hematologic data from previous 2-week inhalation studies conducted in this laboratory with Fischer 344 rats revealed that each of the identified differences were within the historical normal range except PLAT values for the 2319 and 5132 ppm, females and the 5132 ppm males. ▪ Mean WBC counts of males and females from the two highest exposure groups were statistically decreased from control values in an apparent concentration-dependent manner. Examination of mean WBC differential data indicated increased neutrophil:lymphocyte ratios of male and female rats in these two exposure groups. The WBC counts in this study were within the range of historical controls for 29 two-week inhalation studies in this laboratory. ▪ There was no demonstrable microscopic evidence of inflammatory foci in tissues or decreased cellularity of lymphoid organs and bone marrow.
◊ Clinical Chemistry	Clinical chemistry analyses indicated statistically decreased mean values for AP in the 2319 and 5132 males, and the 5132-ppm females when compared to respective control means. These slightly decreased values were not considered toxicologically significant.
◊ Organ Weights	The relative weights of brain, heart, kidneys, liver, lungs, and testes of male rats exposed to 5132 ppm were statistically increased from those of male controls. Similarly, relative weights of heart, liver, and lungs from high exposure group females were increased statistically from the control females. All of the relative organ weight changes were secondary to decreased terminal body weights.
◊ Necropsy findings	Examination of each animal during necropsy did not indicate any exposure-related gross pathologic changes
◊ Histopath-ology	The few microscopic changes seen during the histopathologic examination were considered to be spontaneous events unrelated to exposure. There were no microscopic changes in the brains of high-exposure group rats to account for the transitory clinical signs observed after each exposure..

Conclusions

Remarks Field — The primary effect of dioxolane inhalation exposure to rats was a reduction in white blood cells counts at 2319 or 5132 ppm. Body weight gain was affected at the 5132-ppm exposure level. There was no corresponding myeloid toxicity or inflammatory foci.

Data Quality

- Reliability — Klimisch Code 1. May be used without restriction.

References — 1,3-Dioxolane: A two-Week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company 1989 Study ID K-010634-005

Other — This study is supported by a two-week inhalation study of Dioxolane conducted by BioDynamics for Celanese Corporation. In this study Charles River CD rats (5/sex/group) were exposed for six hours per day, five days per week, for two weeks at concentrations of 0, 984 or 3280 ppm. All animals survived the duration of the study. No treatment related observations were recorded. Body weights, organ weights, clinical chemistry parameters, and macropathology were unremarkable in treated animals. Depressed leukocyte values in male and female exposed animals were reported.[1]

References for supporting studies — A two-week inhalation toxicity study of C-121 (1,3-Dioxolane) in the rat. Bio/Dynamics Inc Project No. 80-7429, June 22, 1981.

Repeated Dose Toxicity, 13-Week Inhalation

Type	Repeated Dose Toxicity, 13-Week Inhalation
● Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Purity >99.78 % by analysis
Method	
● Guideline	This study was conducted to meet the requirements of the United States Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards in 40 CFR part 792; TSCA Health Effects Test Guideline in 40 CFR 798.250; and OECD ISBN 92-64-12367-9 Paris 1982.
● GLP	Yes
● Year	1990
● Species	Rat
● Strain	Fischer 344 Charles River, Kingston NY
● Route of administration	Whole body inhalation of vapor
● Duration of Test	Thirteen weeks plus eight week recovery study
● Doses	0, 298, 1000 or 3010 (Mean, measured concentrations)
● Sex	Male and Female
● Exposure Period	Six hours per day
● Frequency of Treatment	Five days a week Thirteen weeks
● Number of Animals/group	Ten of each sex
● Control Group and Treatment	Ten animals of each sex exposed only to air under the same chamber conditions
● Post-Exposure Observation Period	24 Hours for main groups; eight weeks for satellite recovery groups.

- Statistical Methods
  - ◊ Descriptive statistics (mean and standard deviation), were used to report chamber concentrations, temperature, and relative humidity.
  - ◊ Body weights, absolute and relative organ weights, clinical chemistry, bone marrow myeloid/erythroid ratios and appropriate hematology data for animals to be terminated after 13 weeks were evaluated by Bartlett's test for equality of variances. Clinical chemistry, bone, marrow myeloid/erythroid ratios and appropriate hematology data for satellite animals were evaluated the same way.
  - ◊ WBC differential counts are normally analyzed, in this laboratory, with descriptive statistics. Because effects on the white cell was anticipated based on a two-week study, absolute lymphocyte numbers, absolute neutrophil numbers and myeloid to erythroid cell ratios were evaluated in the following statistical evaluation. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or non-parametric analysis of variance (ANOVA), followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.
  - ◊ Statistical outliers were to be identified by a sequential test and excluded from analysis only for documented scientifically sound reasons that were unrelated to exposure.

Remarks Field for Test Conditions	◊ Age at study initiation	◊ Males: seven weeks ◊ Females: seven weeks
	◊ Number animals of each sex per dose	Ten of each sex per concentration
	◊ Satellite groups	Ten of each sex per dose level and control were exposed for thirteen weeks. Blood samples were taken at four and thirteen weeks of exposure and again at four and eight weeks into the recovery period. Satellite rats were necropsied at the end of the eight-week recovery period.
	◊ Housing	Not specified in report
	◊ Clinical observations performed and frequency	◊ Mortality and gross signs daily ◊ Body weights: weekly ◊ Functional observation battery during the sixth and last week of exposure for main group animals consisting of observations for any unusual conditions with respect to: pupil size, respiration, movement, skin and haircoat, salivation, lacrimation, urine staining, fecal staining, locomotor behavior and responsiveness to touch, noise and tail pinch. ◊ Animals were evaluated for alertness and activity at the beginning and end of each exposure to evaluate sedative effects

◊ Terminal observations	◊ Blood taken for hematology (packed cell volume, hemoglobin, erythrocyte count, total leukocytes platelets, differential leukocyte counts and RBC morphology) and clinical chemistry (urea nitrogen, creatinine, alanine aminotransferase activity, aspartate aminotransferase activity, alkaline phosphatase activity, glucose, total protein, albumin, globulin, total bilirubin, cholesterol, triglycerides, phosphorus, calcium, sodium, potassium and chloride) ◊ Urine samples obtained for urinalysis (bilirubin, glucose, ketones, blood, pH, protein and urobilinogen, specific gravity and microscopic examination of sediment) ◊ Complete gross postmortem examination. ◊ Organ weights for brain, heart, lungs, liver, kidneys, adrenals, thymus, spleen and testes were recorded for each rat.
◊ Histopathology	◊ The following tissues were examined from all control and high-exposure animals only. Adrenals, aorta, bone, bone marrow, brain (cerebrum, brainstem, cerebellum), cecum, cervix, coagulating glands, duodenum, epididymides, esophagus, eyes, gross lesions, heart, ileum, jejunum, kidneys, lacrimal/harderian glands, larynx, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissues, mesenteric lymph node, mesenteric tissues, nasal tissues, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid-gland, tongue, trachea, urinary bladder, uterus, vagina ◊ Microscopic examination of tissues from rats in lower 1,3-dioxolane exposure groups was limited to liver, brain, kidneys, lungs, spleen, bone marrow and gross lesions.

Results

- NOAEL
  - ◊ Males: 1000 ppm
  - ◊ Females: 298 ppm

- LOEAL
  - ◊ Males: 3010 ppm
  - ◊ Females: 1000 ppm

- Mortality

  All animals survived the duration of the study.

- Toxic Responses

Dose	Response
298 ppm	▪ No significant effects
1000 ppm	▪ Reduction in white-blood cell counts for females. ▪ Decrease in spleen weight in females ▪ Increased relative liver weight in females
3010 ppm	▪ Reduction in white-blood cell counts for males and females. ▪ Mild changes in liver of males. ▪ Deceased spleen weights in males and females ▪ Decreased alertness at the end of each exposure ▪ Decreased urine specific gravity

Remarks Field for Results

◊ Body Weights

	Exposure Period Day (n=20)			Recovery Period Day (n=10)		
Males	Day-1	46	86	96	116	152
Control	150	253	299	294	325	323
298 ppm	150	253	300	294	319	314
1000 ppm	147	247	298	287	315	311
3010 ppm	147	257	306	303	332	329
Females						
Control	109	164	182	186	192	187
298 ppm	108	165	183	180	191	186
1000 ppm	108	161	182	183	196	190
3010 ppm	106	158	179	173	189	188

◊ Clinical Signs

◊ Some exposed male and female rats had notable clinical observations including: red swollen eyes, perineal soiling, diarrhea and darkened crusty material around the eyes and/or nares. One male rat exposed to 1000 ppm had persistent redness, swelling and/or crusty material around one eye. This condition, however, was resolved by study termination. The orbital sinus blood sampling procedure may have resulted in some of the observations of crusty material around the eyes. Because of a lack of a dose response relationship and persistence in any of the observations, none were considered to be exposure-related or toxicologically significant

◊ Animals exposed to 3000-ppm dioxolane appeared to have decreased alertness and responsiveness at the end of each exposure. The animals appeared to be fully recovered by the time they were removed from the exposure chamber (approximately 30 minutes after cessation of test material exposure),

◊ Functional Observation Battery

Functional Observational Battery exams were conducted at 6 and 13 weeks. Isolated observations of perineal soiling, decreased activity (one observation) and chromorhinorrhea were noted. These were not thought to be exposure-related due to the low incidence and lack of a dose response.

◊ Hema-tology

◊ WBC

Conc (ppm)	Mean White Blood Cells (x10^3) Week of Study				
Males	4-week	13-week	Recov-4	Recov-8	
Control	10.7±2.1	9.1±1.3	10.2±1.0	10.0±1.2	
298	9.4±2.1	9.6±1.4	8.2±0.8*	9.1±1.1	
1000	8.2±0.8*	8.6±1.0	8.7±1.5*	8.4±0.5*	
3010	5.5±1.0*	6.0±1.0*	8.9±0.9*	8.6±1.2*	
Females					
Control	7.3±1.4	9.2±0.9	7.2±1.3	6.4±1.1	
298	8.7±1.6*	9.1±1.1	6.4±1.1	6.7±0.8	
1000	6.2±0.8	7.8±0.5*	6.1±0.9	6.0±0.6	
3010	4.9±0.9*	5.1±0.6*	6.0±1.0*	5.8±0.6	

◊ Hematology Comments	◊ Although exposure decreased the WBC of rats the magnitude of the decrease at any given time interval was never below the lower limits of historical controls. ◊ The percent eosinophils in 3010 ppm exposed rats was increased relative to controls after 13 weeks of exposure ◊ Small increases in mean platelet counts occurred during the exposure period in some of the 1000 and 3010 ppm groups. There was a single observation of increased platelets in females exposed to 300 ppm at 4 weeks, however, there was no apparent exposure-response relationship and no difference at 13-weeks. The minimal statistical platelet elevations in the 1000 and 3000-ppm groups were within available historical control data; hence these differences were considered to be of minor biological significance. ◊ Sporadic statistically significant variances in red blood cells and hematocrit were in the range of historical controls and did not show concentration-response relationships and are considered of no toxicological significance
◊ Clinical Chemistry	A number of statistically identified differences in various clinical chemistry parameters in exposed male or female rats were identified when compared to their respective controls; however, there was no clear relationship to exposure. Since these parameters were generally within historical control ranges or were not associated with corresponding gross or microscopic effects, most of these clinical chemistry changes were not considered to be diagnostic of a target organ effect. Increased ALT in 3000 ppm exposed rats at 4 weeks, and, in females only at 13 weeks, could be related to the small increases in liver weight. Histological changes were also noted in livers of 3010 ppm exposed males.
◊ Urinalysis	Male and female rats exposed to 3010 ppm for 13 weeks (main study and satellite groups) had statistically decreased urine specific gravity. Repeat urinalyses revealed similar results. The specific gravity alteration was not associated with a morphologic indication of nephrotoxicity. It may represent a physiologic response to repeated exposure to dioxolane vapors.

◊ Organ Weights	◊ Spleen Weights: The mean absolute spleen weight of female rats exposed to 3000 ppm was statistically decreased (27% reduction as compared with control), as were the mean relative spleen weights of males exposed to 3000 ppm (8%) and females exposed to 1000 (11%) and 3000 ppm. The toxicological significance of this observation is uncertain because there were no morphologic alterations observed in the spleen from any animal in the study. It is possible that the decreased spleen weights are associated with reduced WBC counts. ◊ Liver Weights: The mean absolute and relative liver weighs of male and female rats exposed to 3000 ppm dioxolane were statistically increased when compared to control values, as was the mean relative liver weight of females exposed to 1000 ppm. Male rats had minimal microscopic liver changes while females had no morphologic liver alterations. The liver weight increases may be an adaptive physiologic response following repeated exposure. ◊ Other Organs: Sporadic statistically significant variances in other organs were observed but did not show concentration-response relationships or morphological evidence of effect and are considered of no toxicological significance	
◊ Necropsy findings	Gross postmortem examinations revealed no differences among groups which were considered to represent an effect of exposure to the test substance	

◊ Histopathology	◊	Except for liver changes in high-dose male rats, there were no pathologic findings of significance. In male rats exposed to 3000 ppm dioxo1ane, hepatocytes in centrilobular regions of lobules were slightly larger and had more cytoplasmic eosinophilia than controls (incidence 10/10).
	◊	Microscopic examination of bone from exposed and control rats following 13 weeks of exposure indicated that myeloid and erythroid cells were approximately similar in number and, had normal morphology and apparently normal maturation sequences. In addition, there was no cytotoxicity of the microenvironment at the light microscopic level. Therefore, the decreases in WBC counts were not associated with observable histopathologic changes in bone marrow.
	◊	The ratio was lower in male and female rats exposed to 3010 ppm for 13 weeks relative to controls; however, the myeloid/erythroid ratio of this exposure group was similar to that of respective controls after 8 weeks post exposure.

Conclusions

Remarks Field: The primary effect of dioxolane inhalation exposure to rats was a reduction in white blood cells counts at 1000 or 3000 ppm. Rats appeared healthy, body weight gain was not affected and there was no corresponding myeloid toxicity although there was a slight reduction in myeloid cells of the bone marrow at 3000 ppm. Exposure related pathologic effects were limited to slight enlargement of centrilobular hepatocytes of males at 3000 ppm. There was no indication of testicular toxicity based on careful light microscopic examination.

Data Quality

- Reliability: Klimisch Code 1. May be used without restriction.

References: 1,3-Dioxolane: 13-Week Vapor Inhalation Toxicology Study in Fischer 344 Rats. Dow Chemical Company 1990

Other: This study is supported by a 12-month inhalation study in the literature where a concentrations of 2500 mg/m^3 was found not to produce dominant lethal effects or significant signs of toxicity manifest by gross observations or mortality[1]

References for supporting studies: [1] Baraânski B; Stetkiewicz J; Czajkowska T; Sitarek K; Szymczak W Mutagenic and gonadotoxic properties of trioxane and dioxolane Med Pr; Vol 35: P245-55 (1984)

* = p < 0.05

Reverse Mutation Assay - *S. typhimurium*

Type Reverse mutation assay - S. typhimurium

Test Substance 1,3-Dioxolane
CAS Number: 646-06-0

Method

- Guideline: None specified
- GLP: No
- System of Testing: Bacterial
- Year: 1980
- Species/Strain: *S. typhimurium:* TA98, TA100, TA1535, TA 1537, TA1538
- Metabolic activation:
  - ◊ Tested with and without
    - o Rat liver S-9
    - o Aroclor 1245 induced
    - o 0.5 ml S-9 per 100 ml agar plate
- Concentrations tested:
  - ◊ TA98 0, 0.005, 0.010, 0.10, 1, 5, 10, 25, 50 µl/plate
  - ◊ TA100 0, 0.005, 0.010, 0.10, 1, 5, 10, 25, 50 µl/plate
  - ◊ TA1535 0, 0.005, 0.010, 0.10, 1, 5, 10 µl/plate
  - ◊ TA1537 0, 0.005, 0.010, 0.10, 1, 5, 10 µl/plate
  - ◊ TA1538 0, 0.005, 0.010, 0.10, 1, 5, 10 µl/plate
  - ◊ All tested with and without metabolic activation
- Statistical Methods: Evaluation criteria were used as follows:
  - ◊ Strains TA-1535, TA-1537 and TA-1538: If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to three times the solvent control value will be considered to be mutagenic.
  - ◊ Strains TA-98 and TA-100: If the solvent control value is within the normal range, a test material that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-98 and TA-100 will be considered to be mutagenic.

Remarks Field for Test Conditions

- ◊ One replicate
- ◊ Positive Controls
  - o Without activation
    - ▪ Sodium azide: TA100, TA 1535
    - ▪ 9-Aminoacridine: TA1537
    - ▪ 2-Nitrofluorene: TA98, TA1538
  - o With activation
    - ▪ 2-Anthramine: all strains
- ◊ Solvent: none
- ◊ Repeat study: TA-1535 was repeated with and without activation because of low solvent values observed in the initial assay.
- ◊ Evaluation Criteria: Dose-response related increase in revertants specific for historical rates in each strain

Results

- Result — No mutagenic activity under activation or non-activation conditions.
- Cytotoxic Concentration — No significant cytotoxicity at any concentration
- Genotoxic Effects
  - ▪ Not genotoxic under non-activation conditions
  - ▪ Not genotoxic under activation conditions.

Remarks Field for Results

- o Material was soluble in water

Conclusions

Remarks Field

- o Not genotoxic under non-activation conditions
- o Not genotoxic under activation conditions.

Data Quality

- Reliability — Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint. Study was conducted under glp's. Conditions of the assay concerning time of incubation may vary somewhat from current OECD guideline.

References Mutagenicity Evaluation of C-121 (1,3-Dioxolane) in the Ames Salmonella/Microsome Plate Test, Litton Bionetics Study 20988, 2/29/1980 submitted to Celanese Chemical Company.

Other

This study is supported by other bacterial genotoxicity studies specifically:

◊ A negative single-plate 5-strain Salmonella reverse mutations assay with and without metabolic activation by Goodyear[1]

◊ A negative 5-strain Salmonella reverse mutations assay with and without metabolic activation by Hilltop conducted for Ferro under glp conditions.[2]

◊ Published negative 5-strain Salmonella reverse mutations assay with and without metabolic activation.[3]

◊ A combined S. typhimurium and Saccharomyces cerevisiae mutation evaluation conducted by Litton Bionetics for Dow Chemical Company[4].

References for Supporting Studies

1. Mutagenicity Evaluation of 1,3-Dioxolane, Goodyear Tire and Rubber Company. Laboratory report #79-55, 29 November 1979.
2. The Salmonella/Microsomal Assay for Bacterial Mutagenic Activity of Unstabilized 1,3-Dioxolane. Hilltop Research Project No 83-0212-21 10 March, 1983.
3. Kowalski Z, Spiechowicz E, Baraânski B. Absence of mutagenicity of trioxane and dioxolane in Salmonella typhimurium. Mutat Res 136:169-71 (1984).
4. Mutagenicity Evaluation of Compound D8 (1,3-Dioxolane). Litton Bionetics, 1/20/1975 submitted to Dow Chemical Company.

Mutagenic Assay - *S. typhimurium* and *Saccharomyces cerevisiae*

Type Mutagenic assay - *S. typhimurium* and *Saccharomyces cerevisiae*

Test Substance 1,3-Dioxolane
CAS Number: 646-06-0

Method

- Guideline: None specified
- GLP: No
- System of Testing: Bacterial
- Year: 1975
- Species/Strain: *S. typhimurium* TA1535, TA 1537, TA1538
  Saccharomyces cerevisiae strain D4
- Metabolic activation:
  - ◊ Tested with and without induced rat, mouse and monkey homogenates of liver, lung and testis. Animals were induced with a "mixture of polychlorinated biphenyls".
- Concentrations tested:
  - ◊ *Saccharomyces cerevisiae*:
    - o 2.0 and 5.0% in suspension
  - ◊ Salmonella:
    - o 0.75 and 1.50% for suspension incubations
    - o 1.5% for plate tests.
  - ◊ All tested with and without metabolic activation
- Statistical Methods: None specified

- Remarks Field for Test Conditions
  - Duplicate plates, one replicate
  - Salmonella were tested with rat tissues in the suspension test only. Mouse and monkey tissues were used in the plate incorporation technique only.
  - Salmonella and yeast were tested at high and low dose levels using non-activation conditions and activation conditions with rat liver, rat lung and rat testis homogenates.
  - Salmonella were tested under activation conditions with mouse liver, mouse lung and mouse testis homogenates only at the high dose level.
  - Salmonella were tested under activation conditions with monkey liver, monkey lung and monkey testes homogenates only at the high dose level.
  - Yeast were tested only with rat tissue activation and not mouse or monkey.
  - Positive Controls
    - Without activation
      - Ethylmethane sulfonate
      - 2-Nitrofluorene
      - Quinacrine mustard
    - With activation
      - 2-Acetylfluorene
      - Dimethylnitrosamine
  - Solvent: none (except for positive controls)
  - Evaluation Criteria: Dose-response related increase in revertants specific for historical rates in each strain.

Results

- Result: No mutagenic activity under activation or non-activation conditions.

- Cytotoxic Concentration: Fifty percent bacteria survival when tested at 3.0% in the preliminary cytotoxicity test, yeast showed no cytotoxicity at 5% (highest concentration tested).

- Genotoxic Effects
  - Not genotoxic under non-activation conditions
  - Not genotoxic under activation conditions.

Remarks Field for Results

- With the organ homogenates, the positive controls gave significant genotoxic activity only with the liver and testis preparation for the rat, the liver for the mouse and the liver for the monkey.
- Under non-activation conditions, both bacteria and yeast responded as expected to the positive controls.

Conclusions

Remarks Field

- Not genotoxic under non-activation conditions
- Not genotoxic under activation conditions.
- The contribution of the lung and testis homogenates to the genotoxic evaluation of the test substance is questionable since the positive controls did not show significant response except for the rat testis with strain TA-1537
- The main value of this study is the negative response of the yeast as other more robust bacterial genotoxicity tests have been conducted using this test material.

Data Quality

- Reliability

Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although the study was not conducted under glp's.

References

Mutagenicity Evaluation of Compound D8 (1,3-Dioxolane). Litton Bionetics, 1/20/1975 submitted to Dow Chemical Company.

Other

The Salmonella portion of this study is supported by other Salmonella tests providing clear negative results. The key study is listed below.

References for Supporting Studies

Mutagenicity Evaluation of C-121 (1,3-Dioxolane) in the Ames Salmonella/Microsome Plate Test, Litton Bionetics Study 20988, 2/29/1980 submitted to Celanese Chemical Company

***In Vitro* Mammalian Chromosome Aberration Test**

Type	*In Vitro* Mammalian Chromosome Aberration Test
Test Substance	1,3-Dioxolane CAS Number: 646-06-0

Method

• Guideline	None specified
• GLP	Yes
• System of Testing	Non-bacterial
• Year	1985
• Species/Strain	Chinese Hamster Ovary (CHO-WBL), Origin S. Wolff's laboratory UCSF, Litton Bionetics clone, average cell cycle time 12-14 hours.
• Metabolic activation	Tested with and without o Rat liver S-9 o Aroclor 1245 induced o 15 microliters per 10 ml culture
• Concentrations tested	0, 2.0, 3.0, 4.0, 5.0 mg/ml for both non-activation and activation conditions.
• Statistical Methods	Fishers Exact Test for comparisons of treated cultures with pooled controls.

Remarks Field for Test Conditions

◊ Range-finding test to determine cytotoxicity or cell cycle delay used the following conditions:
  - Single culture per dose levels of 0, 1.67 or 5.0 mg/ml for both non-activation and activation conditions. Level of 0.50 was added under activation conditions.
  - Under non-activation conditions there was a 2-hour exposure to test substance followed by 23 hours of incubation prior to addition of colcemid and 2.5 hours of incubation prior to collection.
  - Under activation conditions the same time intervals were employed and fetal-calf serum was omitted.

◊ The aberration test used the following conditions:
  - Duplicate cultures at each dose level of 0, 2.0, 3.0, 4.0, 5.0 mg/ml for both non-activation and activation conditions
  - Solvent, McCoy's 5a culture media.
  - Under non-activation conditions, there was a 7.5-hour exposure followed by 2.5-hr incubation in the presence of colcemid.
  - Under non-activation conditions there was a 2-hour exposure to test material followed by a wash and an 8-hour incubation in the presence of colcemid.
  - 100 Metaphases from each culture were scored for a total of 200 metaphases per dose level.
  - Negative control, McCoy's 5a
  - Positive controls
    - Without activation – Mitomycin C
    - With activation – Cyclophosphamide

Results

- Result

  No increase in the number of aberrations was found at any concentration of test material. Positive controls demonstrated the sensitivity of the test system.

- Cytotoxic Concentration

  Cytotoxicity was not observed up to and including the highest concentration tested of 5 mg/ml which is the highest concentration recommended under the current OECD 473 guideline.

- Genotoxic Effects
  - Not genotoxic under non-activation conditions
  - Not genotoxic under activation conditions.

Remarks Field for Results

- Material was soluble in water
- The number of aberrations per cell, the percent cells with aberrations, and the percent cells with more than one aberration were all similar to appropriate controls under both activation and non-activation conditions.
- Positive controls produced clear significant increases in aberrations as measured by aberrations per cell, the percent cells with aberrations and the percent cells with more than one aberration.

Conclusions

Remarks Field

- Not genotoxic under non-activation conditions
- Not genotoxic under activation conditions.
- Study was well conducted, although the incubation times and exact procedures vary somewhat from the current OECD guideline the consistently low level of aberrations observed at all dose levels both with and without activation and the clear responses of the positive controls strongly support lack of genotoxic activity in this assay.

Data Quality

- Reliability

Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint. Study was conducted under glp’s. Conditions of the assay concerning time of incubation vary somewhat from current OECD guideline.

References

Mutagenicity Evaluation of 1,3-Dioxolane in an In Vitro Cytogenic Assay Measuring Chromosome Aberration Frequencies In Chinese Hamster Ovary (CHO) Cells. Submitted to Ferro Corporation. Litton Bionetics, Inc, Kensington Maryland. LBI Project No. 20990, February 1985

Mouse Lymphoma Forward Mutation Assay

Type	Mouse lymphoma forward mutation assay
Test Substance	1,3-Dioxolane CAS Number: 646-06-0

Method

• Guideline	None specified
• GLP	No
• System of Testing	Non-bacterial
• Year	1985
• Species/Strain	Mouse lymphoma L5178Y TK+/-
• Metabolic activation	Tested with and without ▪ Rat liver S-9 ▪ Aroclor 1245 induced Final concentration of S-9 in cell suspensions was five percent.
• Concentrations tested	0, 750, 1500, 2500, 3000, 4000, 50000 nl/ml for both non-activation and activation conditions.
• Statistical Methods	Simple ratio criteria

Remarks Field for Test Conditions

- ◊ Initial trial under activation conditions discarded because the positive control mutation frequencies were unacceptable.
- ◊ Duplicate plates for counting mutant colonies
- ◊ Solvent, water
- ◊ Negative control, water
- ◊ Positive controls
  - Without activation – EMS (2 levels)
  - With activation – MCA (2 levels)

Results

- Result

No dose-dependent increase in the number of mutants in the absence or presence of metabolic activation was observed. Dioxolane did not induce significant increases in the mutation frequency at the TK locus in L5178Y TK+/- cells. . Positive controls demonstrated the sensitivity of the test system.

- Cytotoxic Concentration

Low dose-dependent cytotoxicity observed under non-activation conditions percent relative growths ranges form 85 % to 61% . Under activation conditions, cytotoxicity was dose-dependent but low, with percent relative growths ranging from 95% to 78%.

- Genotoxic Effects

  ◊ Not genotoxic under non-activation conditions

  ◊ Not genotoxic under activation conditions.

Remarks Field for Results

◊ Material was soluble in water

◊ Under non-activation conditions, the mutant frequency ranged from 12.8 to 22.5 x 10^{-6} without any discernable dose dependency.

◊ Under activation conditions, the mutant frequency ranged from 26.5 to 43.9.5 x 10^{-6} without any discernable dose dependency

◊ The minimum mutation frequency required for a positive response under non-activation conditions based on the control mutation frequencies in this assay was 38 X 10^{-6}.

◊ The minimum mutation frequency required for a positive response under activation conditions, based on the control mutation frequencies in this assay, was 46 X 10^{-6}.

◊ The discarded initial activation assay had positive control values that were considered unacceptably low, although clearly positive. In this discarded test, the mutation frequency for the test substance was also low and similar to that found in the repeated test. The discarded thet supports the final result.

Conclusions

Remarks Field

◊ Not genotoxic under non-activation conditions

◊ Not genotoxic under activation conditions.

◊ Study was well conducted, although there was no glp certification the study appears to have been conducted using a glp-quality protocol in a glp compliant laboratory.

Data Quality

- Reliability

Klimisch Code 2. Reliable with restrictions. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References

Mutagenicity Evaluation of 1,3-Dioxolane in the Mouse Lymphoma Forward Mutation Assay. Submitted to Ferro Corporation. Litton Bionetics, Inc, Kensington Maryland. LBI Project No. 20989, April 1985

Other

Mammalian Cell Transformation Assay, *in vitro*

Type	Mammalian Cell Transformation Assay, *in vitro*
Test Substance	1,3-Dioxolane CAS Number: 646-06-0

Method

• Guideline	None specified
• GLP	No
• Year	1981
• Cell Type	C3H 10T-1/2 clone 8
• Concentrations tested	0, 1, 10, 20, 50, 100, 500, 1000, 2000, 5000, 10000, 20000 and 40000 µg/ml
• Statistical Methods	None used

Remarks Field for Test Conditions	◊ Dose selection based on initial cytotoxicity testing using plating efficiency as a metric. ◊ A set of replicates was exposed in T-25 flasks and a set of replicates was exposed in flat 60 mm culture dishes. ◊ Incubation of plates carried out in sealed Mason jars to prevent volatilization ◊ T-25 flasks were sealed to prevent loss of test material. ◊ Twenty-four hour incubation time with test substance ◊ Cells washed after 24-hour incubation with test substance ◊ Cells fed with fresh media 24 hours after exposure was complete and every three days until the 14-day point then weekly (with 10%FCS supplementation) for the duration of the 38 day culture period. ◊ At the 14-day interval, four to six T-25 flasks at each concentration and three to five dishes were stained and examined. ◊ At the 38-day interval, two to four T-25 flasks at each concentration and three or four dishes were stained and examined. ◊ High-concentration levels (>2000 µg/ml) of T-25 flasks at the 38-day interval were contaminated with a fungus in the initial assay and were run a second time about 2 months later. In this repeat assay, six T-25 flasks were incubated as before for 38 days at each concentration level from 1 to 20,000 µg/ml, stained and examined. The data varied from the initial assay in that there was a much higher transformation rate in the positive controls (BP 5 or 6 times higher) and the transformation rate for the test substance was much higher at the repeated concentration levels. The negative control still showed no transformed colonies. ◊ Positive control: Benzpyrene @ 2.5, 1.0 or 0.5 µg/ml ◊ Colonies examined macroscopically and microscopically. ◊ 300 cells plated per flask or dish
	◊ Differences from Guideline: The study is similar to the Draft OECD Guideline "*In Vitro* Syrian Hamster Embryo Cell Transformation Assay". The major differences from this guideline are the type of cells used and the incubation times employed. It was similar or superior in other respects including number of dose levels employed.

Results

• Result	◊ Report authors conclude that Dioxolane is a "weak positive" in this cell-transformation assay. ◊ The data suggest transforming activity but there are issues of concern with the data. The poor reproducibility, the lack of clear dose-concentration relationships, the test material sometimes giving results similar to positive controls and at other times no response, and the lack of high-concentration toxicity data make it difficult to conclude that this assay is positive. ◊ A more conservative interpretation of the data is "Not interpretable"

- Cytotoxic Concentration: Cytotoxicity observed above 5,000 ug/ml. There was no valid measure of cytotoxicity given for the 38-day examinations

- Genotoxic Effects: Not interpretable

Remarks Field for Results

◊ The majority of transformed foci were classified as "type II" rather than "type III" This is defined as follows:

  - Type I. Foci composed of monolayer cells that are more densely packed than the background cells. This type is not considered malignant and is not scored.

  - Type II. Foci show massive piling up into virtually opaque multilayers. The cells are only moderately polar, thus criss-crossing is not pronounced. Fifty percent of Type II foci have been shown to be malignantly transformed.

  - Type III. Foci are composed of highly polar, fibroblastic, multilayered, criss-crossed arrays of densely stained cells. Eighty-five percent of Type III foci have been shown to be malignantly transformed.

	14-Day Foci/plate		38-Day Foci/plate		Repeat 38-day
Conc (µg/ml)	T-25	Dishes	T-25	Dishes	T-25
0	0.0	0.0	0.0	0.0	0
1	0.0	0.0	0.0	0.0	2.8
10	0.0	0.0	0.0	0.0	2.8
20	0.0	0.0	0.0	0.0	3.7
50	0.0	0.0	0.0	0.0	3.7
100	0.0	0.0	1.0	0.75	4.7
500	0.0	0.0	0.3	1.33	4.0
1,000	0.0	0.0	1.5	1.25	5.0
2,000	0.6	-	C	-	-
5,000	0.0	0.0†	C	2.0	4.66
10,000	0.5†	0.0†	C	2.25	1.2
20,000	0.0††	0.0††	C	0.25	0
40,000	-	0.0††	-	-	
BP 0.5	0.3	0.75	0.5	6	3.0
BP 1.0	1.0	1.0	1.25	12	6.0
BP 2.5	-	-	-	-	7.0
	- = Not run, † = Cytotoxic, , †† = Highly Cytotoxic C = contaminated, BP = Benzpyene Positive Control				

Conclusions

Remarks Field

The report authors concluded that these results represent a weak positive; however, the reviewer (who prepared this summary) disagrees with this interpretation and judges the study not interpretable with regard to genotoxic activity.

Data Quality

- Reliability

Klimisch Code 3. Reliable with severe restrictions, study design, conduct and reporting are considered reliable to address the endpoint but the data are not clearly supportive of the author's conclusions.

References

An Assay of Cell Transformation and Cytotoxicity in C3H 10T 1/2 Clonal Cell Line for the Test Chemical C-121 (1,3-Dioxolane). University of Minnesota Environmental Pathology Laboratory, Authors Garry and Nelson, 27 August 1981.

Other

This study is supported by a study using BALB/C-3T3 cell cultures. This study, however, reported Dioxolane to give a clear negative response in cell transformation.

Reference for supporting study

Evaluation of 1,3-Dioxolane in the In Vitro Transformation of BALB/C-3T3 Cells Assay. Litton Bionetics, Inc, Project 20992, January 1985. Submitted to Ferro Corporation

Mammalian Cell Transformation Assay, *in vitro*

Type	Mammalian Cell Transformation Assay, *in vitro*
Test Substance	1,3-Dioxolane CAS Number: 646-06-0

Method

• Guideline	None specified
• GLP	Not specified (report was reviewed by the quality assurance unit but did not specifically state that the test was conducted under glp)
• Year	1985
• Cell Type	◊ BALB/C-3T3 ◊ Subclone 14 of Clone 1-13 from Takeo Kakunaga
• Concentrations tested	0, 1000, 4000, 8000, 12000 and 15000 nl/ml
• Statistical Methods	Bailey's modification of Student's t-test was applied to calculate the statistical significance of the positive control and test material transforming activity.
Remarks Field for Test Conditions	◊ Positive control: 2.0 micrograms/ml MCA ◊ Seventy-two hour treatment time followed by washing and continued incubation for four weeks with re-feeding twice a week. ◊ Treatments carried out in sealed 25 cm^2 flasks to prevent volatilization. ◊ Doses based on preliminary cytotoxicity range-finding test. ◊ 30,000 cells plated per culture vessel ◊ 18 Cultures per dose level (lower n in Results table due to contaminated flasks) ◊ Simultaneous cytotoxicity test conducted under same conditions as transformation assay to verify the toxicity of test material to cells.

Preliminary Cytotoxicity Concentration (nl/ml)	Average Number of Colonies per Flask	Relative cell Survival (%)
0	94.3	100.0
1000	92.0	97.6
2000	86.3	91.6
4000	74.0	78.5
8000	55.0	58.3
16000	4.0	4.2
Simultaneous Cytotoxicity Test		
0	59.7	100.0
1000	51.0	85.4
4000	46.3	77.6
8000	29.7	49.7
12000	8.3	14.0
15000	0.0	0.0

◊ Differences from Guideline

The study is similar to the draft OECD Guideline "*In Vitro* Syrian Hamster Embryo Cell Transformation Assay". The major differences from this guideline are the type of cells used and the incubation times employed. It was similar or superior in other respects including number of dose levels, replicates, cytotoxicity testing and MTD selection. The current study protocol was shown to be sensitive by the response of the positive controls.

Results

- Result

No increase in the number of transformed colonies or transformed foci was noted at any concentration of test material. Positive controls demonstrated the sensitivity of the test system

- Cytotoxic Concentration

Severe cytotoxicity observed above 12000 micrograms/ml, moderate dose-dependent cytotoxicity observed at 1000 microgram per ml and above. Data in table above

- Genotoxic Effects

Not genotoxic under these conditions

Remarks Field for Results

The test material was water soluble and precipitation was not recorded.

Results	Transforming Activity		
Concentration (nl/ml)	Log_{10} (x+l) (mean±sem)	(n)	Foci/Culture average
0	0.050±.027	(18)	0.12
1000	0.050±.027	(18)	0.12
4000	0.067±.030	(18)	0.17
8000	0.050±.027	(18)	0.12
12000	0.067±.030	(18)	0.17
15000	0.075±.034	(16)	0.19
MCA (2µg/ml)	0.602±.039	(18)	3.00**
** p<0.01; MCA = 3 Methylcholanthrene			

Conclusions

Remarks Field

No genotoxic activity. The transforming activities of all five doses of Dioxolane were comparable to the spontaneous background average rate of 0.12 foci/culture vessel and there was no evidence of a dose-related response with the test material. This range of test material treatments corresponded to 0 to 85% survival in the simultaneous colony survival assay. Although the survival assay, using only 200 cells, suggested there would be no surviving cells with test material treatments at 15000 nl/ml, some cells survived in the transformation assay where 30,000 cells were plated per culture vessel. Therefore, concentrations of 1,3-dioxolane from 1,000 to 15,000 nl/ml were evaluated as being nontransforming to Balb/C-3T3 cells.

Data Quality

- Reliability

Klimisch Code 2. Reliable with restrictions, study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References

Evaluation of 1,3-Dioxolane in the In Vitro Transformation of BALB/C-3T3 Cells Assay. Litton Bionetics, Inc, Project 20992, January 1985. Submitted to Ferro Corporation

Other

This study is supported by a previous study using C3H 10T-1/2 cell cultures. This study reported Dioxolane to give a weak positive response in cell transformation but is considered uninterruptible by the writer of the robust summary.

Reference for Supporting Study

An Assay of Cell Transformation and Cytotoxicity in C3H 10T 1/2 Clonal Cell Line for the Test Chemical C-121 (1,3-Dioxolane). University of Minnesota Environmental Pathology Laboratory, Authors Garry and Nelson, 27 August 1981

Mouse Micronucleus Assay

Type Mouse micronucleus assay

Test Substance 1,3-Dioxolane
CAS Number: 646-06-0

Method

- Guideline None specified, Meets or exceeds OECD 474 guideline
- GLP Yes
- Year 1989
- Species/Strain Mice/ICR Harlan Sprague Dawley
- Sex Mice of each sex
- Route of Administration IP Injection in Corn Oil
- Doses 0, 525, 1050 and 2100 mg/kg
- Exposure Period 24, 48 and 72 hours
- Statistical Methods The incidence of micronucleated polychromatic cells was compared to controls with a significant increase relative to the negative control set at the p≤0.05 level using Kastenbaum-Bowman Tables*

Remarks Field for Test Conditions

◊ Age at Study Initiation: Six to eight weeks

◊ Number of animals per dose: Five of each sex at each dose-time sacrificewere planned, more animals were dosed due to mortality. High-dose mortality was unexpectedly high and a supplemental group of 15 animals of each sex was dosed at 2100 mg/kg. Of these supplemental animals only four of each sex survived to the scheduled sacrifice at 72 hours. There were insufficient surviving high-dose animals to sacrifice five per dose at the high dose. The resulting dosing and sacrifice of animals is shown below:

	Number of Animals Sacrificed at Each Time					
Dose	24 hours		48 hours		72 hours	
(mg/kg)	M	F	M	F	M	F
0	5	5	5	5	5	5
525	5	5	5	5	5	5
1050	5	5	5	5	5	5
2100	5	5	3	5	4	4

◊ Control Groups and Treatment

- Negative Control: Corn Oil
- Positive Control: Triethylenemelamine in water at 0.25 mg/kg

◊ Clinical Observations

- All low and mid-dose animals appeared normal. High-dose animals showed several clinical observations indicating toxicity including prostration, lethargy, irregular breathing, and ruffled fur.

◊ Criteria for Evaluating Results

- Negative control must not exceed an incidence of 5/1000 micronucleated polychromatic cells.
- A positive result is indicated if the incidence of micronucleated polychromatic cells is significantly increased relative to the negative control ($p \le 0.05$, Kastenbaum-Bowman Tables)
- 1000 polychromatic erythrocytes scored for each animal.

◊ Criteria For Selection of MTD

- The high dose was set as 80% of the LD_{50} (7 day). A preliminary toxicity study was conducted with results shown below. The LD_{50} (7 day) was calculated by probit analysis to be approximately 2603 mg/kg.

Prelim Tox Study	Seven Day Mortality	
IP Dose (mg/kg)	Males	Females
Contol	0/5	0/5
1576	0/5	0/5
2048	1/5	0/5
2663	5/5	3/5
3462	5/5	5/5
4500	4/5	5/5

◊ Differences from Current OECD Guideline

This study meets all requirements of the current OECD 474 guideline. The study used more dose levels and time points than indicated in the guideline. As there was a previous positive study in the literature the study design was especially robust.

Results

- Effect on PCE/NCE ratio by dose level by sex.

	PCE/Total Erythrocytes					
Dose	24 Hours		48 Hours		72 Hours	
(mg/kg)	M	F	M	F	M	F
0	0.59	0.59	0.62	0.62	0.62	0.66
525	0.53	0.57	0.62	0.64	0.54	0.65
1050	0.53	0.60	0.58	0.67	0.43	0.54
2100	0.31	0.44	0.26	0.41	0.37	0.35
TEM	0.57	0.57	-	-	-	-

- Genotoxic Effects: Negative

- NOAEL: 1050 mg/kg for bone marrow toxicity as measured by the PCE/NCE ratio

Remarks Field for Results

◊ Induction of Micronucleated Cells

	Micronucleated Cells/ 1000 PCE					
Dose	24 Hours		48 Hours		72 Hours	
(mg/kg)	M	F	M	F	M	F
0	0.2	0.2	0.2	0	0.2	0
525	0.2	0.2	0.2	0	0.4	0.6
1050	0.2	0.2	0	0.4	0	0
2100	0.6	0	0.3	0.4	0	0
TEM	18.4	20.8	-	-	-	-

◊ Mortality

- All low and mid-dose mice survived to sacrifice.
- Mortality at high dose was males 23/35 and females 21/35

Conclusions

Remarks Field

◊ There was no induction of micronucleated polychromatic cells associated with administration of Dioxolane.

◊ There was significant induction of micronucleated polychromatic cells associated with administration of the positive control.

◊ Dioxolane showed no genotoxicity in this assay under these conditions.

◊ The highest dose of dioxolane was toxic to the animals including the bone marrow.

Data Quality

- Reliability — Klimisch Code 1. Reliable without restriction.

References

Micronucleus Cytogenetic Assay in Mice. Test article C-121 [1,3-Dioxolane]. Microbiological Associates Inc., Study No. T9034.122, December 28, 1989

* Kastenbaum, M and K Bowman. Tables for determining the statistical significance of mutation frequencies. Mutation Res. 9:527-549 (1970)

Other

An earlier study in the literature reported Dioxolane was positive in the micronucleus assay. In this report mice were given dioxolane ip in two doses at 24 hr intervals. Dioxolane at doses from 1500 mg/kg to 6000 mg/kg caused a significant increase of micronucleated polychromatic erythrocytes in bone marrow of mice as compared to the negative control value.

Reference for Supporting Study

Przybojewska B, Dziubautowska E, Kowalski Z. Genotoxic effects of dioxolane and trioxane in mice evaluated by the micronucleus test. Toxicol Lett 21:349-52 (1984)

Dominant Lethal, Oral Dosing

Type	Dominant Lethal, Oral Dosing
Test Substance	Dioxolane CAS Number: 646-06-0

Method

• Guideline	None specified but basically in accord with OECD 478
• GLP	No
• Year	1984
• Species	Rat
• Strain	Albino, Wistar
• Route of administration	Oral gavage
• Doses	0, 580, 1160 mg/kg
• Sex	Male
• Number of Animals/group	10
• Vehicle	Water

Remarks Field for Test Conditions

◊ Age at Study Initiation	3 ½ to 4 months; weight 300 to 320 g
◊ Doses	0, 580 and 1160 mg/kg/day
◊ Dosing	Males only
◊ Dosing Schedule	Five days a week
◊ Dosing Duration	Eight weeks
◊ Mating Interval	Weekly
◊ Mating Ratio	2:1 females:males
◊ Variations from OECD 478 Protocol Guideline	Guideline suggests that the number of males should be sufficient that 30-50 pregnant rats be evaluated at each time interval. In this study, 13 to 20 pregnant females were evaluated at each time; however, more time periods than typical were evaluated in this study. Current guideline suggests three dose levels; only two were used in this study.

Results

- Result — No evidence of dominant lethal effect

- Dominant Lethal Rate

	Week (Dominant Lethals per Female)							
Dose	1	2	3	4	5	6	7	8
0 mg/kg	0.84	0.83	1.45	1.29	0.89	1.11	0.93	0.67
580 mg/kg	0.73	1.31	0.94	0.89	0.75	0.78	1.06	1.29
1160 mg/kg	0.94	0.64	0.88	1.23	0.56	0.46	0.47	0.67

- Number of deaths at each dose level

Dose	Mortality
0 mg/kg	0/10
580 mg/kg	1/10
1160 mg/kg	1/10

Remarks Field for Results

◊ Time of death

580 mg/kg group — one animal during fifth week
1160 mg/kg group — one animal during third week

◊ Clinical Signs

Behavior and appearance did not differ to any significant degree from controls

◊ Body Weights

Dose (mg/kg)	Body Weight gain (percent control gain)
580	47%
1160	22%

◊ Organ Weights

Absolute and relative weights of liver, kidney and spermatic vesicle were increased at both dose levels.

◊ Necropsy and Microscopic Findings

Necropsy finding are not discussed. Histopathology was performed on the testes. Necrosis of the seminiferous epithelium was reported in 1/10 controls, number unspecified in the 580 mg/kg group and 3/9 high dose males.

◊ Target Organs: Testis considered target by histopathology but no change observed in fertility. Other organ weights were altered but there is no histopathology for confirmation. No changes in testes weight resulted from dosing.

◊ Criteria for Dominant Lethal Effect: Investigators used early resorptions as a measure of dominant lethal effect; however, neither implants per female, live fetuses per female nor preimplantation loss per female was affected by treatment.

Conclusions

Remarks Field: Study documentation is good for a published article. The study appears to have been well conducted and is similar to current OECD guideline. Based on reduction in body weigh gains, organ weight changes and histopathology all dose levels produced clear signs of toxicity indicating the study is valid regarding use of a minimally toxic dose as the high-dose level. There was no evidence of a dominant lethal effect.

Data Quality

- Reliability: Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

References

Baranski, B; Stetkiewicz, J. Evaluation of Mutagenic and Gonadotoxic Properties of Trioxane and Dioxolane. Medycyna Pracy:35 245-255 (1984)

Other

Dominant Lethal, Inhalation

Type	Dominant Lethal, Inhalation
Test Substance	Dioxolane CAS Number: 646-06-0
Method	
• Guideline	None specified conducted basically in accord with OECD 478
• GLP	No
• Year	1984
• Species	Rat
• Strain	Albino, Wistar
• Route of administration	Whole body inhalation
• Doses	0, 2500 mg/m^3
• Sex	Male
• Number of Animals/group	14
• Vehicle	Air
• Duration of Dosing	12 Months
• Statistical Methods	Kruskal Wallis Test followed by non-parametric tests for groups with and without normal distribution.

Remarks Field for Test Conditions	◊ Age at Study Initiation	3 ½ to 4 months; weight 300 to 320 g
	◊ Doses	0 and 2500 mg/m^3
	◊ Dosing	Males only
	◊ Dosing Schedule	Five days a week, five hours a day.
	◊ Dosing Duration	12 Months
	◊ Mating Interval	Once at end of study for one week
	◊ Mating Ratio	2:1 females:males

◊ Variations from OECD 478 Protocol Guideline: Guideline suggests that the number of males should be sufficient that 30-50 pregnant rats be evaluated at each time interval. In this study, 18 to 20 pregnant females were evaluated. Current guideline suggests three dose levels; only one was used in this study.

◊ Other: Concentration of test material measured by chromatography using a published procedure.

Results

- Result: No evidence of dominant lethal effect

- Dominant Lethal Rate

	Effect (per female)					
Dose	Number Pregnant	Live Fetuses	Dead Implants	Number Implants	Corpora Lutea	Preimplantation loss
0 mg/m3	20	11.4	0.6	12.0	13.7	1.8
2500 mg/m3	18	11.1	0.17	11.3	13.6	2.3

- Number of deaths at each dose level

Dose	Mortality
0 mg/m^3	0/14
2500 mg/m3	0/14

Remarks Field for Results

◊ Time of death: No deaths

◊ Clinical Signs: Not reported

◊ Body Weights: Not reported

◊ Organ Weights: Not reported

◊ Necropsy and Microscopic Findings

Not clearly reported. It was stated that for the “rats exposed to Dioxolane the frequency of necrosis of the spermicidal epithelium was much higher than for the control group rats”; but the incidence or other details was not given. Lydig cell lesions were also mentioned but it was also stated that the treated and control groups were not different regarding this lesion.

◊ Target Organs

None reported.

◊ Criteria for Dominant Lethal Effect

Investigators used early resorptions as a measure of dominant lethal effect; however, neither implants per female, live fetuses per female nor preimplantation loss per female was affected by treatment.

Conclusions

Remarks Field

Study documentation is good for a published article. The study appears to have been well conducted and the protocol is similar to the current OECD guideline. Based on reduction in body weigh gains, organ weight changes and histopathology, all dose levels produced clear signs of toxicity indicating the study is valid regarding use of a maximum tolerated dose. There was no evidence of a dominant lethal effect.

Data Quality

- Reliability

Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards. Study design and reporting meets current EPA/OECD guidelines with minor exceptions.

References

Baranski, B; Stetkiewicz, J. Evaluation of Mutagenic and Gonadotoxic Properties of Trioxane and Dioxolane. Medycyna Pracy:35 245-255 (1984)

Other

One Generation Reproduction Study (Drinking Water, low dose)

Type	One Generation reproduction Study
Test Substance	1,3-Dioxolane CAS Number: 646-06-0
Method	
• Guideline	None, generally follows OECD 415
• GLP	No
• Year	1976
• Species	Rat
• Strain	Charles River strain albino rats (Charles River Wilmington Mass)
• Route of administration	Oral, drinking water
• Doses	0.01, 0.03 or 0.1 % in drinking water
• Sex	Males and females
• Control Group and Treatment	Received water without test substance
• Frequency of Treatment	Seven days a week
• Duration of Test	21 Weeks
• Premating Exposure Period for Males	90 Days
• Premating Exposure Period for Females	None for F1a generation
• Statistical Methods	Not described other than that a 95% confidence interval was used to evaluate statistical significance.
• Number of Animals/group	10
• Vehicle	Water

Remarks Field for Test Conditions	◊ Age at Study Initiation	Not specified
	◊ Dosing	Dosed water was available seven days a week and 24 hours a day.
	◊ Test Material Preparation	Test material was prepared weekly and added to the water bottle. Bottles were changed weekly.
	◊ Dosing Duration	Dosing started 90 days prior to mating for males. Females were dosed starting with the mating period and throughout gestation and lactation.
	◊ Mating Interval	Mating continued for 15 days or until conception was confirmed.
	◊ Examinations	Females were examined daily for evidence of copulation.
	◊ Mating Ratio	Two females to one male
	◊ Culling	Litters of more than 10 pups were reduced to that number by random sacrifice on the fourth day of the lactation period.
	◊ Weaning	Litters were weaned 21 days postpartum
	◊ Variations from current OECD 415 Protocol Guideline	Significant variations include: ○ Number of pregnant females was about 10 rather than about 20. ○ Females were treated from the beginning of the mating period rather than from two weeks prior to mating. ○ Necropsies and microscopic evaluations were not conducted on animals.
	◊ Comments	The test material is volatile and significant loss occurs from open systems. Experience indicates that it is stable in solution in closed systems. The weekly preparation and changing of water bottles likely resulted in a small loss of test material concentration as the week progressed. It is unlikely that this affects the results.

Results

- Result
  - ◊ No treatment-related effects were noted among the indices used to evaluate reproductive performance.
  - ◊ No significantly significant differences were noted between the control and test group with regard to the number of pups delivered stillborn or viable or cannibalized and viable at lactation days 1, 4, 12, or 21.
  - ◊ No treatment-related effects were noted among the progeny survival indices.
  - ◊ No treatment-related effects in body weight were noted for the F1a progeny at lactation days 0, 1, 4, 12 or 21.
  - ◊ No treatment-related body weight differences were noted for the female parental rats.

- NOAEL 0.1% in drinking water for parental and F1a generations

Remarks Field for Results

◊ Mortality One gravid high-dose female died during the F1a gestation period. Necropsy examination revealed no gross abnormalities

◊ Parental Data F1a Litter

	Indices for Parental Parameters F1a Litter (percent)				
Dose	Female Mating	Female Fecundity	Male Fertility	Female Fertility	Parturition
0	76.9	100	100	100	100
0.01	60.0	100	100	90	88.9
0.03	47.1	87.5	87.5	70	85.7
0.10	84.6	90.0	100	100	90.0

◊ Parental Body Weights Parental body weight gains were unaffected by treatment with the test article.

◊ Progeny Data F1a Litter

		Survival Indices*		# Pups Weaned		21-day mean Body Weights (g)	
Dose	Live Birth Index	4-Day	21-Day	Male	Female	Male	Female
0	100	94.9	96.7	46	42	42	40
0.01	97.6	97.5	82.1	35	39	20	39
0.03	96.1	90.5	80.9	26	44	29	42
0.10	100	81.9	94.7	41	40	30	40

◊ F1a Exam
- All delivered pups appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.
- The significantly reduced mean body weights of 21-day male pups in the 0.01 dose group is considered spurious due to lack of dose response correlation.

◊ Clinical Signs — None noted

Conclusions

Remarks Field — Adverse effects recorded were limited to a significant but likely spurious difference between low-dose treated and control animals in decreased male pup body weight (low-dose group on lactation day 21). No significant differences were observed between treated and control animals in the following: maternal mortality, mating and fertility indices, male and female fertility, incidence of parturition, gross abnormalities in pups, number of pups delivered stillborn or viable, or cannibalized (lactation days 1, 4, 12 or 21), survival indices, female pup body weight, and female parental body weight. It is concluded that 0.1% in drinking water represents a LOAEL for parental and F1a generations.

Data Quality

- Reliability — Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References — One-Generation Reproduction Study with 1,3-Dioxolane in Drinking Water in Albino Rats Results of the FO Generation. Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries March 26 1976.

Other — These results are supported by an inhalation study at 125 ppm in which no significant effects on parental or pup parameters were observed. [1,2]

References for supporting studies

1. Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and F1a Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries March 1, 1976.

2. Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and F1b Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries April 13, 1976.

Definitions	○ Female Mating Index = 100(number copulations[i]/number estrus cycles required) ○ Female Fecundity Index = 100(number pregnancies/number copulations) ○ Male Fertility Index = 100(number sires/number males mated) ○ Female Fertility Index = 100(number of pregnancies/number of females mated) ○ Parturition Index = 100(number of parturitions/number pregnancies) ○ Survival Index, 4-day = 100(number pups viable at lactation day 4/number of viable pups born) ○ Survival Index, 21-day = 100(number pups viable at lactation day 21/number of viable pups at lactation day 4) i) Only one copulation counted per estrus cycle.

One Generation Reproduction Study (Drinking Water, high dose)

Type	One Generation reproduction Study with F1a and F1b litters
Test Substance	1,3-Dioxolane CAS Number: 646-06-0
Method	
• Guideline	None, generally follows OECD 415
• GLP	No
• Year	1975
• Species	Rat
• Strain	Charles River strain albino rats (Charles River Wilmington Mass)
• Route of administration	Oral, drinking water
• Doses	0.5 or 1.0 % in drinking water
• Sex	Males and females
• Control Group and Treatment	Received water without test substance
• Frequency of Treatment	Seven days a week
• Duration of Test	25 Weeks
• Premating Exposure Period for Males	90 Days for males used to produce F1a pups, males used to produce F1b pups were untreated.
• Premating Exposure Period for Females	None for F1a generation, dams for F1b generation were treated for approx 67 days prior to mating. This started with the first mating period and throughout gestation, lactation and a 10-day rest period after weaning of F1a litter before mating a second time.
• Statistical Methods	Not described other than that a 95% confidence interval was used to evaluate statistical significance.
• Number of Animals/group	10
• Vehicle	Water

Remarks Field for Test Conditions	◊ Age at Study Initiation	Not specified
	◊ Dosing	Dosed water was available seven days a week and 24 hours a day.
	◊ Test Material Preparation	Test material was prepared weekly and added to the water bottle. Bottles were changed weekly.
	◊ Dosing Duration	Dosing started 90 days prior to mating for males. Females were dosed starting with the mating period and throughout gestation, lactation and a 10-day rest period after weaning of F1a litter before mating a second time.
	◊ Mating Interval	Mating continued for 15 days or until conception was confirmed.
	◊ Examinations	Females were examined daily for evidence of copulation.
	◊ Mating Ratio	Two females to one male
	◊ Culling	Litters of more than 10 pups were reduced to that number by random sacrifice on the fourth day of the lactation period.
	◊ Weaning	Litters were weaned 21 days postpartum
	◊ Production of F1b Generation Pups	Due to the severe effects on offspring and possible males in the F1a generation, treated dams were rested for 10 days after F1a pups were weaned, and mated with unexposed proven breeders. Females were exposed to test substance during the 10-day interval after weaning F1a pups. Neither dams nor males were exposed after the F1b mating interval started.
	◊ Sacrifice, Gross Pathology and Organ Weights	Following weaning of the F1b litter, all surviving female rats were necropsied. Early deaths were also necropsied. Weights of adrenal glands, gonads, pituitary glands and uterus were recorded at necropsy. Fixed tissues (adrenals, ovaries, pituitary glands, uterus and all apparent lesions) were sectioned and stained with H&E for microscopic examination.
	◊ Variations from current OECD 415 Protocol Guideline	Significant variations include: ○ Number of pregnant females was about 10 rather than about 20. ○ Females were treated from the beginning of the mating period rather than from two weeks prior to mating in the F1a mating.

◊ Comments

The test material is volatile and significant loss occurs from open systems. Experience indicates that it is stable in solution in closed systems. The weekly preparation and changing of water bottles likely resulted in a small loss of test material concentration as the week progressed. It is unlikely that this affects the results especially in light of the significant effects produced by treatment.

Results

- Result
  - ◊ Mating with males pretreated 90 days with dioxolane to produce the F1a litter:
    - Treated groups copulated less frequently than controls.
    - Fewer pregnant treated animals delivered that than controls.
    - One high-dose male failed to impregnate either female he was paired with and was considered sterile
    - There was a decrease in the number of pups delivered by the high-dose group.
    - Both treated groups showed an increase in the number of stillborn pups.
    - There was reduced survival in progeny from treated animals as compared to controls. Especially the high-dose 24-hour survival, which was zero percent. Reduced survival indices were noted for the 0.5% group at each time interval examined.
    - Dam's body weight was significantly less than control's for the high-dose group on postpartum days 1 and 4.
  - ◊ Mating with proven breeders to produce the F1b litter
    - There was a decrease in the fecundity index and in the parturition index in the test groups.
    - There was a lower female fertility index for the exposed groups.
    - Gross necropsy and histopathology of treated dams was unremarkable, although was a tendency for chances in the ovaries.
    - Male fertility (unexposed males) was 100%
    - Mating index was unaffected
    - All pups appeared normal and no significant differences were noted with respect to number of pups born, stillborn, cannibalized, born viable or at lactation days 1, 4, 12, and 21. Pup body weight was unaffected.

- NOAEL — Not found, effects at all concentrations tested for F0 and F1 generations
- LOAEL
  - F0 (P) Generation: 0.50%
  - F1a Generation: 0.50%
  - F1b Generation: Not applicable since no concurrent exposure

Remarks Field for Results

◊ Mortality: One high-dose male died during the last week of the 90-day treatment period, necropsy revealed the cause of death due to respiratory problems not related to test article exposure.

◊ Parental Data F1a Litter

	Indices for Parental Parameters F1a Litter (percent)				
Dose (%)	Female Mating	Female Fecundity	Male Fertility	Female Fertility	Parturition
0	81.8	100	100	90.0	88.9
0.5	40.9	88.9	100	80.0	50.0
1.0	35.3	100	75	75.0	50.0
Number pregnant: control=9; 0.5% = 8, 1.0% = 6 (one high-dose male was found to be sterile)					

◊ Parental Body Weights Dams F1a

	Mean weights of dams on day indicated						
Dose	G-15	Part	PP-1	PP-4	PP-12	PP-21	PP-28
0	375	369	364	366	345	352	359
0.5	398	362	344	367	377	371	365
1.0	359	331	324[a]	316[a]	338	338	334
G-15 = gestation day 15, Part= day of parturition, PP=postpartum day a = significant at $p < 0.05$							

◊ Progeny Data F1a Litter

	Number of pups that were			
Dose	Delivered	Stillborn	Cannibalized	Viable
0	55	2	0	53
0.5	45	6	0	39
1.0	14	7	1	6

◊ F1a data

		Survival Indices*		# Pups Weaned		21-day mean Body Weights (g)	
Dose (%)	Live Birth Index	4-Day	21-Day	Male	Female	Male	Female
0	96.4	92.5	70.2	15	18	47	47
0.5	86.7	61.5	41.7	8	2	42	44
1.0	42.9	0.0	0.0	0	0	-	-

◊ F1a Exam — All pups delivered appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

◊ Clinical Signs — None noted

◊ Mortality During the F1b Mating and Gestation — One high-dose female died post-mating. Necropsy showed non-gravid female with pyometra.

◊ F1b Litter Data, Parental Data

Indices for Parental Parameters F1b Litter (percent)					
Dose (%)	Female Mating	Female Fecundity	Male Fertility	Female Fertility	Parturition
0	64.3	88.9	100	80	100
0.5	64.3	66.7	80	60	83.3
1.0	66.7	50.0	75	50	75

◊ Dam Body Weights — Body weights of dams were unaffected by prior treatment with dioxolane during the mating, parturition and lactation phase of the F1b litter production

◊ F1b Litter Data, Pup Data

	Number of pups that were: [total (mean per dam)]			
Dose	Delivered	Stillborn	Cannibalized	Viable
0	78 (9.8)	2(0.3)	0(0.0)	76(9.5)
0.5	43(8.9)	2(0.3)	0(0.0)	41(8.2)
1.0	24(8.0)	0(0.0)	0(0.0)	24(8.0)

◊ F1b Litter Data, Pup Data

		Survival Indices*		# Pups Weaned		21-day mean Body Weights (g)	
Dose (%)	Live Birth Index	4-Day	21-Day	Male	Female	Male	Female
0	97.4	92.1	86.9	30	23	45	44
0.5	95.3	92.7	69.4	10	15	57	51
1.0	100	91.7	72.7	9	9	59	44

◊ F1b Pup Exam — All pups delivered appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

◊ Clinical Signs

None noted

◊ Gross Pathology

No significant differences were noted between control and test group parental females upon gross pathologic examination.

◊ Dam Organ Weights

	Organ weights, absolute in grams, relative in g/100g bw					
	Adrenals		Ovaries		Uterus	
Dose	abs	rel	abs	rel	abs	rel
0	0.082	0.0208	0.072	0.0179	0.713	0.1803
0.5	0.076	0.0184	0.075	0.0184	0.797	0.1909
1.0	0.071	0.0172	0.065	0.0156	0.686	0.1658

Adrenals, ovaries and uterus weights showed a tendency to reduced weight with increasing dose. None were statistically different from control but the (n) was relatively small. Other organs showed no tendency to decreased weight with increasing dose of dioxolane.

◊ Histopathology of Dams

◊ All weighed organs were examined microscopically . No histopathological alterations attributable to the effects of the test material were observed. In treated rats, the hilus of the ovary was unusually prominent and the amount of ovarian parenchyma appeared deficient.

◊ Although not considered significant by the examining pathologist, the incidence of ovarian focal "leutinization of stroma" was 0/10, 1/10 and 3/8 for control, low dose and high dose animals respectively.

Conclusions

Remarks Field

Drinking water exposure of rats at 0.5 or 1.0% produced clear adverse effects on reproduction. In the f1a mating, it could not be determined if effects were attributable primarily to exposure of males, females or both. Survival of high-dose pups was especially affected. In the second exposure using untreated proven male breeders, the effects were less severe suggesting that there was a male contribution to the reproductive toxicity. This conclusion is clouded, however, since the females were not exposed to test article during mating, gestation or lactation during the production of this second litter. It is clear that the females were still affected and therefore the female was an affected sex regarding reproductive toxicity of dioxolane.

Data Quality

- Reliability — Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References — One-Generation Reproduction Study with Dioxolane in Albino Rats, Results of the FO Generation. Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries July 21, 1975.

One-Generation Reproduction Study with Dioxolane in Drinking Water of Albino Rats Results of the FO Generation Females and Proven Breeder Males Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries February 16, 1976.

Other — The perinatal mortality is supported by a the study of prenatal and postnatal development published by Sitarek et al. in which an increased perinatal death rate was reported after dosing dioxolane at 1150 mg/kg/day, every other day, from day 2-20 of gestation.

References for supporting studies — Sitarek K, Baranski B, Berlinska B. The effect of maternal exposure to dioxolane on prenatal and postnatal development in rats. Pol J Occup Med Environ Health 5:159-66 (1992).

Definitions

- Female Mating Index = 100(number copulations[i]/number estrus cycles required)
- Female Fecundity Index = 100(number pregnancies/number copulations)
- Male Fertility Index = 100(number sires/number males mated)
- Female Fertility Index = 100(number of pregnancies/number of females mated)
- Parturition Index = 100(number of parturitions/number pregnancies)
- Survival Index, 4-day = 100(number pups viable at lactation day 4/number of viable pups born)
- Survival Index, 21-day = 100(number pups viable at lactation day 21/number of viable pups at lactation day 4)

i) Only one copulation counted per estrus cycle.

One Generation Reproduction Study, Inhalation

Type	One Generation reproduction Study with F1a and F1b litters
Test Substance	1,3-Dioxolane CAS Number: 646-06-0

Method

• Guideline	None, generally follows OECD 415
• GLP	No
• Year	1975
• Species	Rat
• Strain	Charles River strain albino rats (Charles River Wilmington Mass)
• Route of administration	Inhalation, whole body exposure
• Doses	125 ppm
• Sex	Males and females
• Control Group and Treatment	Untreated controls, under same chamber housing conditions, same number of animals per group
• Frequency of Treatment	Five days a week (seven hour a day exposure)
• Duration of Test	24 Weeks
• Premating Exposure Period for Males	90 Days for males used to produce F1a pups, 120 days for males (different males) used to produce F1b pups.
• Premating Exposure Period for Females	None for F1a generation, dams for F1b generation were treated for approx 35 days during the F1a mating and gestation, then remained unexposed for approximately 35 days prior to mating and the second exposure period.
• Statistical Methods	Not described other than that a 95% confidence interval was used to evaluate statistical significance.
• Number of Animals/group	10
• Vehicle	Air

Remarks Field for Test Conditions	◊ Age at Study Initiation	Not specified
	◊ Dosing	Inhalation, only water was supplied during exposure. Exposures were conducted in the same chamber being used for an ongoing chronic study of Dioxolane at the laboratory.
	◊ Test Material Preparation and Measurement	The inhalation chambers were supplied with vapors of dioxolane generated by bubbling nitrogen gas through test material in a gas-washing bottle. These vapors were diluted with filtered-conditioned air to achieve the desired concentration. Vapor concentration was measured by gas chromatography hourly during the first two weeks of the study then twice daily for the remainder of the study.
	◊ Dosing Duration	○ P males for F1a generation were treated starting 90 days prior to mating. These males were sacrificed after mating. ○ P males for F1b generation were selected from the population in an ongoing chronic study and had been treated for 120 days prior to mating. ○ Dams for the F1a litter production were exposed starting with the 15-day mating period and through gestation. Exposure was stopped a day or two before parturition. ○ Dams for the F1b litter production were exposed about 35 days previously during F1a litter production. They were then not exposed during delivery and lactation. After weaning, exposure was conducted again during the mating and gestation period of the F1b litter production. Exposure was stopped a day or two before delivery
	◊ Mating Interval	Mating continued for 15 days.
	◊ Mating Ratio	Two females to one male
	◊ Culling	Litters of more than 10 pups were reduced to that number by random sacrifice on the fourth day of the lactation period.
	◊ Weaning	Litters were weaned 21 days postpartum
	◊ Production of F1b Generation Pups	After weaning the F1litters of pups, the dams were mated with different males selected from an ongoing chronic study.

◊ Sacrifice, Gross Pathology and Organ Weights

Males used to produce the F1a generation were sacrificed after mating and necropsied. Organ weights were recorded for brain, gonads, heart, kidneys, liver, lungs and spleen. A list of 31 tissues were removed and fixed for sectioning and staining including testes, seminal vesicles, prostate and pituitary.

◊ Variations from current OECD 415 Protocol Guideline

Significant variations include:

- Number of pregnant females was about 10 rather than about 20.
- Females were treated from the beginning of the mating period rather than from two weeks prior to mating in the F1a mating.
- Only one dose group utilized.

Results

- Result

Treatment did not significantly affect any measured reproductive parameter. There was a tendency toward an overall reduction in the number of pups born and weaned in both the F1a and F1b litters of the dosed group but this was not statistically significant.

- NOAEL

Authors conclusions:

◊ F0 (P) Generation 125 ppm

◊ F1a Generation 125 ppm

◊ F1b Generation 125 ppm

- LOAEL

Not stated in the report, apparently > 125 ppm

Remarks Field for Results

◊ Mortality During the F1a Mating and Gestation

No mortality observed

◊ Parental Data F1a Litter

	Indices for Parental Parameters, F1a Litter (percent)				
Dose	Female Mating	Female Fecundity	Male Fertility	Female Fertility	Parturition
0	57.9	54.5	100	60.0	100.0
125	64.7	54.5	80	60.0	100.0
Number pregnant: control=6/10 125 ppm = 6/10					

◊ Parental Body Weights Dams F1a

	Mean weights of dams on day indicated						
Dose (ppm)	G-15	Part	PP-1	PP-4	PP-12	PP-21	PP-28
0	409	366	361	359	379	373	348
125	377	348	343	346	354	359	339
G-15 = gestation day 15, Part= day of parturition, PP=postpartum day							

◊ Progeny Data F1a Litter

	Number of pups that were			
Dose (ppm)	Delivered	Still-born	Cannibalized	Viable
0	65 (10.8)[a]	2	0	63 (10.5)
125ppm	43 (7.2)	1	0	42 (7.0)
a = mean per litter in parentheses				

◊ F1a data

F1a		Survival Indices*		# Pups Weaned		21-day mean Body Weights (g)	
Dose	Live Birth Index	4-Day	21-Day	Male	Female	Male	Female
0	96.9	93.7	98.1	23	28	49	46
125	97.7	97.6	97.4	23	14	49	50

◊ F1a Exam

All pups delivered appeared outwardly normal. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

◊ Clinical Signs

None noted

◊ Organ weights P-males

Males used to produce the F1a litter showed no difference in absolute or relative organ weights for brain, gonads, heart, kidneys, liver, or spleen. Absolute lung weighs were reduced by 8 percent in treated males and the relative lung weighs were reduced by 11 percent (statistically significant $p<.05$).

◊ Histo-pathology

Microscopic examination of tissues from the males used to produce the F1a litter was unremarkable. The organ weight changes in lungs did not have a histopathological correlate. No adverse effects on reproductive tissues were noted.

◊ Mortality During the F1b Mating and Gestation

No mortality observed.

◊ F1b Litter Data, Parental Data

Indices for Parental Parameters F1b Litter (percent)					
Dose (ppm)	Female Mating	Female Fecundity	Male Fertility	Female Fertility	Parturition
0	92.9	69.2	100	90	100
125	50.0	60.0	80	60	100
Number pregnant: control = 9/10, 125 ppm = 6/10					

◊ Dam Body Weights

Body weights of dams were unaffected by prior treatment with dioxolane during the mating, parturition and lactation phase of the F1b litter production

◊ F1b Litter Data, Pup Data

	Number of pups that were: [total (mean per dam)]			
Dose (ppm)	Delivered	Still-born	Cannibalized	Viable
0	91(10.1)	8(0.9)	0(0.0)	83(9.2)
125	64(10.7)	2(0.3)	0(0.0)	62(10.3)

◊ F1b Litter Data, Pup Data

		Survival Indices*		# Pups Weaned		21-day mean Body Weights (g)	
Dose (ppm)	Live Birth Index	4-Day	21-Day	Male	Female	Male	Female
0	91.2	91.6	67.7	29	13	50	43
125	96.9	91.9	81.1	20	23	46	45

◊ F1b Pup Exam

All pups delivered appeared outwardly normal with the exception of a control female pup that had microphthalmia of the right eye. An examination of each pup for external abnormalities was conducted at weaning and all pups were judged free of abnormalities.

◊ Clinical Signs

None noted

◊ Other — The report contains no indication that the pups or dams were necropsied at sacrifice, it is assumed they were sacrificed without necropsy.

◊ Comment — Although treatment did not significantly affect any measured reproductive parameter, there was a tendency toward an overall reduction in the number of pups born and weaned in both the F1a and F1b litters of the dosed group. The lack of statistical significance may partly be a function of the low numbers of animals employed in the study. The possibility that the 125 ppm exposure represents a LOAEL for reproduction in the F1a and F1b litters cannot be excluded.

Conclusions

Remarks Field

Treatment of male and female albino rats with Dioxolane by inhalation at 125 ppm did not significantly affect any measured reproductive parameter. The tendency toward an overall reduction in the number of pups born and weaned in both the F1a and F1b litters of the dosed group suggests that the statistical significance could have been reached with a larger (n). Although 125 ppm was considered to be NOAEL by the authors of the study, the possibility cannot be excluded that 125 ppm represents a LOAEL for reproduction under these conditions.

Data Quality

- Reliability — Klimisch Code 2. Study design, conduct and reporting are considered reliable to address the test endpoint although not conducted in accord with glp standards.

References

This study report was split into two documents by the testing laboratory. As it was a unified experiment with the same dams, both were combined for preparing this robust summary. The two reports are listed below.

- Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and F1a Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries March 1, 1976.

- Single Generation Reproduction Study with the Vapors of Dioxolane in Albino Rats Results of the FO and F1b Generations, Status Report. IBT No. 663-05562 Industrial Bio-Test Laboratories, Inc. Submitted to PPG Industries April 13, 1976.

Other

These results are supported by two oral studies from the same laboratory in four reports (reports were split by litter).

References for supporting studies	1. Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxolane in Drinking Water in Albino Rats, Results of the F0 Generation. (1975), EPA Document No. 878213523, Fiche No. OTS0205848. 2. Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxolane in Drinking Water in Albino Rats, Results of the F0 Generation and Proven Breeder Males. (1975), EPA Document No. 878213524, Fiche No. OTS0205848 3. Industrial BIO-TEST Laboratories, Inc.; One-Generation Reproduction Study With Dioxolane in Drinking Water in Albino Rats, Results of the F0 Generation. (1975), EPA Document No. 878213525, Fiche No. OTS0205848 4. Industrial BIO-TEST Laboratories, Inc.; Single Generation Reproduction Study With the Vapors of Dioxolane in Albino Rats, Results of the F0 and F1a Generations, Status Report. (1976), EPA Document No. 878213527, Fiche No. OTS0205848
Definitions	○ Female Mating Index = 100(number copulations[i]/number estrus cycles required) ○ Female Fecundity Index = 100(number pregnancies/number copulations) ○ Male Fertility Index = 100(number sires/number males mated) ○ Female Fertility Index = 100(number of pregnancies/number of females mated) ○ Parturition Index = 100(number of parturitions/number pregnancies) ○ Survival Index, 4-day = 100(number pups viable at lactation day 4/number of viable pups born) ○ Survival Index, 21-day = 100(number pups viable at lactation day 21/number of viable pups at lactation day 4) i) Only one copulation counted per estrus cycle.

Developmental Toxicology

Type	Developmental Toxicology
Test Substance	1,3-Dioxolane CAS Number: 646-06-0 Hoechst Celanese Lot UN1166 Purity verified by analysis

Method

• Guideline	U.S. Environmental Protection Agency Toxic Substances Control Act Test Guidelines - 798.4900
• GLP	Yes
• Year	1991
• Species	Rat
• Strain	Charles River Crl:CD®BR VAF/Plus
• Route of administration	Oral gavage in corn oil
• Doses	0, 125, 250, 500 and 1000 mg/kg
• Sex	Female, pregnant
• Exposure Period	Days 6 to 15 of pregnancy
• Frequency of treatment	Daily
• Control Group	Corn Oil
• Duration of test	15 days

- **Statistical Methods**

Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution(1).
Maternal body weights, body weight changes, gravid uterine weights and feed consumption values, and litter averages for fetal body weights, percent male fetuses, percent resorbed conceptuses per litter, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Homogeneity of Variances(2) and the Analysis of Variance(3), when appropriate (i.e., Bartlett's Test was not significant ($P>0.05$)). If the Analysis of Variance was significant ($P<0.05$), Dunnett's Test(4) was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($P<0.05$)], the Kruskal-Wallis Test(5) was used, when less than or equal to 75% ties were present; when more than 75% ties were present, Fisher's Exact Test(6) was used. In cases in which the Kruskal-Wallis Test was statistically significant ($P<0.05$), Dunn's Method of Multiple Comparisons(7) was used to identify the statistical significance of the individual groups. All other Caesarean-sectioning data were evaluated using the procedures previously described for the Kruskal-Wallis Test(5).

1. Snedecor, G.W. and Cochran, W.G.. Variance test for homogeneity of the binomial distribution. Statistical Methods, 6th Edition, Iowa State University Press, Ames, pp. 240-241.(1967).
2. Sokal, R.R. and Rohlf, F.J. Bartlett's test of homogeneity of variances. Biometry, W.H. Freeman and Co., San Francisco, pp. 370-371(1969).
3. Snedecor, G.W. and Cochran, W.G. Analysis of Variance. Statistical Methods, 6th Edition, Iowa State University Press, Ames, pp. 258-275(1967).
4. Dunnett, C.W. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1129(1955).
5. Sokal, R.R. and Rohlf, F.J. Kruskal-Wallis Test. Biometry, W.H. Freeman and Co., San Francisco, pp. 388-389 (1969).
6. Siegel, S. Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, New York, pp. 96-104(1956).
7. Dunn, O.J. Multiple comparisons using rank sums. Technometrics 6(3):241-252(1964).

Remarks Field for Test Conditions

◊ Age at Study Initiation
- Females were approximately 93 days old at first dosing
- Males were approximately 24 weeks old when bred to the virgin females

◊ Number of animals per group
- 25 mated presumed-pregnant females per dose group

◊ Test Substance Preparation and Analysis
- Test substance was prepared weekly at 4 concentrations (prepared twice) was analyzed for content of test material and each batch was analyzed seven days later to establish stability.

◊ Analysis and Stability

Target Conc. (mg/gm)	Day-1 Conc (mg/gm)		7-Day Conc (mg/gm)	
	Prep 1	Prep 2	Prep 1	Prep 2
25	26	28	19	27
50	52	56	48	56
100	100	114	94	112
200	198	223	190	219

◊ Vehicle

- Corn oil

◊ Clinical Observation Performed and Frequency

- Viability observed at lest twice daily.
- Additional observations for clinical signs of test substance effects, abortions, premature deliveries and deaths were also made immediately prior to intubation (days 6 through 15 of presumed gestation) and approximately one hour post-dosage. These additional observations were made once daily during the post-dosage period (days 16 through 20 of presumed gestation).
- The body weights of the rats were recorded at least once weekly prior to mating. Body weights and feed consumption values were recorded on day 0 and on days 6 through 20 of presumed gestations.

◊ Mating Procedures

Following the acclimation period (approximately two weeks), 160 healthy virgin female rats were placed in cohabitation with 160 breeder male rats (one male rat per female rat). The remaining 20 virgin female rats were placed in cohabitation with breeder male rats that had mated during the first night of the cohabitation period. Female rats with spermatozoa observed in a vaginal lavage or a copulatory plug observed *in situ* were considered to be at day 0 of presumed gestation and assigned to individual housing. Female rats mated by the same male rat were assigned to different dosage groups.

◊ Maternal Parameters Assessed During Study	Body weight, feed consumption and clinical signs during life. A gross necropsy was conducted on each rat. The intact gravid uterus was excised and weighed. The number and placement of implantation sites, early and late resorptions, live and dead fetuses, and the number of corpora lutea in each ovary were recorded. Uteri from rats that appeared nonpregnant were stained with ammonium sulfide to confirm pregnancy status
◊ Fetal Parameters Assessed During Study	Litter size, placental weight, gross malformations, fetal body weight, sex ratio, body cross sections, skeletal examination. Live fetuses were sacrificed by immersion in the appropriate fixative. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations by using a variation of Wilson's sectioning technique. The remaining fetuses in each litter were cleared, stained with alizarin red S(7) and examined for skeletal alterations.
◊ Organs Examined at Necropsy	List not provided, stated in protocol that visceral organs that are abnormal will be noted and retained
◊ Rationale for Dose Selection	The doses were selected based on a 14-day gavage study in non-pregnant rats of the same strain.

Results

- NOAEL & LOEL for Maternal Toxicity
  - ◊ NOAEL = 250 mg/kg/day
  - ◊ LOEL = 500 mg/kg/day Body weight gain

- NOAEL & LOEL for Developmental Toxicity
  - ◊ NOAEL = 500 mg/kg/day
  - ◊ LOEL = 1000 mg/kg/day Reduced fetal body weights and gross external, soft tissue and skeletal malformations or variations occurred in the 1000 mg/kg/day dosage group fetuses.

- Actual Doses Received: Not calculated, but very close to the target doses of 0, 125, 250, 1500 and 1000 mg/kg/day

- Maternal data

No rats died during the conduct of this study. No clinical or necropsy observations were caused by dosages of the test substance as high as 1000 mg/kg/day. The 500 and 1000 mg/kg/day dosages of Dioxolane caused weight loss in dams on days 6 to 7 of gestation. This initial weight loss was significant ($P<0.05$) for the 1000 mg/kg/day dosage group, and average maternal body weight gain was reduced for this group for the entire dosage period (calculated as days 6 to 16 of gestation), as compared with the control group value. Dosages of 250 and 500 mg/kg/day slightly reduced maternal feed consumption and the 1000 mg/kg/day dosage of the test substance produced a more marked reduction in absolute (g/day) and slightly reduced relative (g/kg/day) maternal feed consumption values during the dosage period as compared with the control group values. Significantly decreased ($P<0.05$) absolute feed consumption values occurred for the 500 and 1000 mg/kg/day dosage groups on days 6 to 7 of gestation. Administration of the 1000 mg/kg/day dosage of Dioxolane significantly decreased ($P<0.05$) absolute maternal feed consumption values for the entire dosage period.

- Fetal data

◊ Cesarean Data

Litter size was comparable in all groups. No Caesarean-sectioning parameters were affected by administration of 1,3-Dioxolane to the dams at dosages as high as 1000 mg/kg/day. The averages for corpora lutea, implantation sites, litter sizes, live fetuses and resorptions, and the numbers of dams with any resorptions were comparable for the five dosage groups. No litters consisted of only resorbed conceptuses

◊ External and visceral effects

The 1000 mg/kg/day dosage of the test substance resulted in significant increases in the litter and fetal incidences of externally evident tail malformations and vertebral malformations interrelated with the tail malformations and septal defects in the heart. The gross external and soft tissue malformations that were considered effects of the 1000 mg/kg/day dosage of the test substance were eight fetuses (from seven litters) in the 1000 mg/kg/day dosage group litters that had malformed tails. These malformations differed in severity and included constrictions of the tail, thread-like tail, short tail and absent tail; thread-like tail occurred for four fetuses (from four different litters) in this dosage group. Soft tissue examination revealed three fetuses (from three litters) at 1000 mg/kg/day dosage group litters that had ventricular septal defects as their only alteration. One high-dose fetus was found with a cleft palate.

◊ Major skeletal defects

Skeletal examination of fetuses from the 1000 mg/kg/day group revealed increases in vertebral malformations associated with the tail malformations (five fetuses †† from four litters †† had absent caudal vertebrae) and other alterations in vertebral and rib ossification that were probably interrelated with the more severe effects evident in the fetuses with visible tail malformations. These alterations included "scrambling" or reduced ossification of centra in the thoracic vertebrae (asymmetric, bifid, unilateral or absent ossification, fused centrum and arch), the lumbar vertebrae (fused centrum and arch, centra that were bifid or not ossified, and arches that were small or not ossified), absent sacral and caudal vertebrae and rib-vertebral malformations. These vertebral malformations and delays in ossification occurred in 4, 0, 0, 11 and 36†† fetuses from 3, 0, 0, 7 and 16 †† litters in the 0, 125, 250, 500, and 1000 mg/kg/day dosage groups, respectively. Of these alterations, significant increases ($P<0.01$) in the litter and fetal incidences occurred for thoracic vertebrae with bifid, unilaterally ossified or not ossified centra, lumbar vertebra with a bifid centrum, and absent caudal vertebrae. In the 1000 mg/kg/day group, the litter average for the average number of ossified metacarpal bones per fetus was significantly decreased ($P<0.01$) for this dosage group, a delay in ossification that is expected because of the significantly decreased ($P<0.01$) fetal body weight that also occurred for this group.

◊ Fetal and Litter Effects

	Dose Group				
Effect	0	125	250	500	1000
Litters evaluated	25	24	25	23	24
Fetuses evaluated	405	388	382	362	376
Litters with altered fetuses	9	10	5	8	21††
Fetuses with any alteration	17	10	6	16	52††
Percent fetuses with any alteration per litter	4.43	2.44	1.52	4.16	13.78††
Tail malformations, fetuses	0	0	0	0	8††
Tail malformations, litters	0	0	0	0	7 ††
Heart, ventricular septal defect	0	0	0	0	3††
Vertebral scrambling, fetuses	4	0	0	11	36 ††
Vertebral scrambling, litters	3	0	0	7	16 ††
Vertebral/rib malform., fetuses	0	0	0	0	2
Vertebral/rib malform litters	0	0	0	0	2
Sternebrae alterations, fetuses	7	5	2	0	5
Sternebrae alterations, litters	4	5	2	0	4

◊ Ossification

Statistically significant reductions in the average number of ossified metacarpals per fetus occurred for the 500 and 1000 mg/kg/day dosage group litters, as compared with the control group values. This observation was considered biologically important and an effect of the test substance for only the 1000 mg/kg/day dosage group because: 1) no other statistically significant dosage-dependent delays in ossification occurred for the 500 mg/kg/day dosage group; 2) the values for the 500 and 1000 mg/kg/day dosage groups did not demonstrate expected differences reflecting the magnitude of the difference in the dosages provided (the 500 and 1000 mg/kg/day dosage group litters had averages of 3.12 ± 0.18 † and 3.10 ± 0.21 †† ossified metacarpal sites per fetus, values that are not biologically different); and 3) the statistical significance of the value for the 500 mg/kg/day dosage group reflected the relatively small standard deviation that occurred for this group, as compared with the higher values for the standard deviations of the other dosage groups.

Remarks Field for Results

Food Consumption

Food Consumption per dam (gm/day)					
	Dose Level (mg/kg/day)				
Time	0	125	250	500	1000
Days 6-7	22.2	21.7	19.2	18.6†	18.2†
Days 6-16	22.4	23.6	21.3	21.4	20.7†
Days 6-20	23.8	25.0	23.1	23.4	23.0

† = $p<0.05$

	Dose Group (mg/kg/day)				
Parameter	0	125	250	500	1000
Pregnancies	25/25	25/25	25/25	23/25	24/25
Corpora lueta (per liter)	19.6	21.2	19.8	20.5	21.5
Implantations (per liter)	17.0	17.2	16.7	16.8	17.1
Liter size	16.2	16.2	15.3	15.7	15.7
Live fetuses	405	388	382	362	376
Dead fetuses	0	0	0	0	0
Early resorptions (total)	19	23	36	24	31
Late resorptions (total)	0	1	0	0	3
Resorptions per liter	4.4	5.8	8.6	6.5	8.3
Males %	47.8	52.0	50.0	52.1	49.5
Body weight (mean grams)	3.38	3.54	3.46	3.48	3.16††

Conclusions

Remarks Field

Based on the results of this study, Dioxolane is not considered a specific developmental toxin. The developmental NOEL was found to be 500 mg/kg/day while the maternal NOEL was found to be 250 mg/kg/day. Neither deaths nor adverse clinical signs were observed in any of the animals. No adverse effects are anticipated for the conceptus in the absence of maternal toxicity.

The maternally-toxic 1000 mg/kg/day dosage of the test substance significantly reduced ($P<0.05$ to $P<0.01$) fetal body weights for male and female fetuses and for the combined sexes. This dosage of the test substance also significantly increased ($P<0.05$ to $P<0.01$) the litter and fetal incidences of externally evident tail malformations, vertebral malformations interrelated with the tail malformations and septal defects in the heart, and significantly reduced ($P<0.01$) the litter average for the average number of ossified metacarpal bones per fetus, an expected delay in ossification related to the significantly decreased ($P<0.01$) fetal body weights.

Data Quality

- Reliability — Klimisch Code 1. Reliable without restriction, study meets GLP standards and/or most requirements

References — 1,3-Dioxolane: Oral Developmental Toxicity Study In Crl:CDOBR VAF/Plus's Presumed Pregnant Rats, Argus Research Laboratories, Inc. 905 Sheehy Drive Horsham, Pennsylvania 19044 Argus report # 508-002, May 9, 1991, submitted to Hoechst Celanese Corporation.

Other — A subsequent study was published which supports this result. In this study Dioxolane was administered by gavage every other day from days 8-20 of gestation as an aqueous solution daily doses of 0.14, 0.58 or 1.15 g/kg/day. Dioxolane administration was not associated with increased embryo or fetus intrauterine death rates or congenital defects at any dose level. The mid (0.58 g/kg) and high dose (1.15 g/kg) were reported to be associated with dose-related delays in fetal development and the high dose showed clear maternal toxicity. The developmental and maternal NOEL were both judged to be 0.58 g/kg/day under these conditions of every other day dosing.

References for supporting studies — Sitarek K, Baranski B, Berlinska B. The effect of maternal exposure to dioxolane on prenatal and postnatal development in rats. Pol J Occup Med Environ Health 5:159-66 (1992).

† = $p \leq 0.05$, †† = $p \leq 0.01$